

The
Decomposition of Woolscour and
Fellmongery Sludges

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ABSTRACT

This thesis investigated two industrial sludges, a woolscour sludge and a fellmongery sludge. The sludges represent material removed during the clarification of effluent arising, respectively, from the washing of greasy wool and the dehairing of sheepskins for leather manufacture. The aims of the study were to (i) determine the degree to which the woolscour and fellmongery sludges decompose, (ii) to describe the probable constraints upon sludge decomposition and (iii) to assess the likely consequences of sludge decomposition in a soil system.

Most experiments were performed at a microcosm scale. The extent to which the woolscour and fellmongery sludges decomposed and the constraints upon their decomposition were determined using net-N mineralisation. An assessment of the consequences of sludge decomposition included leachable mineral N and an estimation of the amount of microbial biomass (MB) in sludge-amended soils. The MB was estimated using the fumigation-extraction method.

Woolscour sludge mineralised only 9 % of its initial total N (i-TN) during decomposition at 22 °C, however N mineralisation increased dramatically to 40 % i-TN when the sludge was incubated at 50 °C. Fellmongery sludge mineralised 52 % i-TN at 22 °C, but showed no net-N mineralisation at 43 or 50 °C. When the sludges were decomposed in an anaerobic atmosphere, the fellmongery sludge mineralised only 15 % i-TN after 108 days. Anaerobic conditions did not greatly affect the decomposition of woolscour sludge, which mineralised 5 % i-TN in 108 days. It is suggested that different pools of the soil MB were involved in the decomposition of the woolscour sludge and the fellmongery sludge.

One of the consequences of the rapid decomposition of fellmongery sludge was the leaching of water-soluble mineral N. During the decomposition of surface-applied fellmongery sludge (at a rate equivalent to 350 kg N ha⁻¹) to leaching columns (5-cm x 15-cm height), 25 to 39 % of the applied N was leached as mineral N in 100 days. Most of the mineral N leached was present as nitrate (68 to 93 %), which exceeded 10 mg nitrate L⁻¹ of leachate for 45 to 80 days.

The amendment of soil with woolscour and fellmongery sludge, at a rate equivalent to 200-kg N ha⁻¹ and incubated in microcosms, caused a dramatic decrease in

the MB, such that the level of MB was below detection using the fumigation-extraction technique after a year of soil incubation. When soil was amended with woolscour and fellmongery sludges in the field and the soil sampled ten months post-amendment, the MB had also decreased, although the effect was weaker than that seen in the microcosm study. In addition, less N was mineralised from woolscour and fellmongery sludges decomposing in their respective sludge-amended soil, than when decomposed in non-amended soils. Together, these results indicate that the application of woolscour and fellmongery sludges to soil has the potential to significantly impede nutrient cycling through the reduction of microbial function.

Fellmongery sludge showed inconsistent results for its value as a fertiliser. On the one hand, fellmongery sludge-amended soil that had been heavily leached for 105 days prior to ryegrass seeds being planted, produced significantly more grass (165 mg (dry weight; dw)) than from the non-amended, leached control soil (106 mg (dw)), after 50 days of grass growth. The enhancement of grass growth was seen more strongly when already established ryegrass received a fresh application of fellmongery sludge, at a rate equivalent to 350-kg N ha⁻¹. In 20 days of grass growth, the amended soil produced 375 mg (dw) of ryegrass, whereas the non-amended soil produced 103 mg (dw). These results indicated that fellmongery sludge could provide nutrients that promoted the growth of ryegrass. However, on the other hand, ryegrass harvested from fellmongery sludge-amended soil was enriched with nitrate (5.9 % of the TN was nitrate for grass grown on the amended-soil but only 1.5 % from non-amended soil). The ryegrass growing on the soil re-amended with the fellmongery sludge all died after the 20 days harvest, whereas the grass on the non-amended columns continued to grow. In addition, cucumber seeds failed to germinate in the presence of fresh fellmongery sludge.

Two case studies were carried out concurrently with this research. In the first, it was found that the long-term practice (>15 years) of burying excess woolscour sludge in soil had decreased the MB-N of woolscour waste-amended soil from 4.0 % to 1.4 % of soil N. In the second case study, it was found that mixtures of woolscouring and fellmongering wastes that had received 90 days of composting treatment were unsuitable media in which to grow cucumber seedlings, which indicated that the composting treatment of these wastes had been unsuccessful. The unsuccessful formation of compost during these trials was considered to be due, mainly, to technical problems.

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TABLE OF ABBREVIATIONS

AN	Ammonium nitrate
BOD	Biological oxygen demand
CC	Commercial compost
COD	Chemical oxygen demand
dw	Dry weight
EPA	Environmental Protection Agency (USA)
FE	Fumigation-extraction
i-TC	Initial total C
i-TN	Initial total N
MB	Microbial biomass
RMA	Resource Management Act
SEM	Scanning electron micrograph
SRM	Seed-raising mix
SS	Suspended solids
TC	Total C
TN	Total N
TS	Topsoil
WRONZ	The Wool Research Organisation of New Zealand
WSCA	The Water and Soil Conservation Act, 1967

1 INTRODUCTION

This thesis is divided into seven sections. In the first section, the Introduction, the processes of woolscouring and fellmongering are described, as are some of the disposal methods used for organic wastes. A model of nutrient cycling, especially for N, is introduced using plant litter decomposition as an example. Sewage sludge is used to provide information about the decomposition of an animal-based waste material, against which it is speculated that the decomposition of woolscour and fellmongery sludges might be compared. The first section finishes with a description of the woolscour and fellmongery sludges used for the experimental work here.

In the second chapter, the Material and Methods, the hypotheses of the thesis are stated, followed by the experimental details used to test them. The third chapter, the Results, together with the fourth chapter, the Discussion, presents experimental results and whether the hypotheses were supported by the evidence collected. Sections five and six are two case studies, which while incomplete as judged by usual scientific criteria, provide valuable “real life” sections to this thesis. Chapter seven is the Conclusion and draws together the hypotheses tested and suggests some implications to the disposal of woolscour and fellmongery sludges.

1.1 INTRODUCTION

The disposal of some domestic and industrial wastes may have detrimental effects on the environment unless well-informed decisions are made about where, when and how these wastes will be disposed. Ignorance about the chemical and biological nature of organic wastes may lead to inappropriate disposal, inadequate utilisation of waste material or cleaning up wastes that don't represent a significant hazard, all of which can detract from effective waste management. For industries associated with large cities, municipal sewage works can usually handle most liquid wastes entering industrial sewers (Alloway and Ayres, 1997). Industry is charged (in dollar terms) on the volume, suspended solids (SS), biological oxygen demand (BOD) and chemical oxygen demand (COD) of their effluents (Brach *et al.*, 1990; Buljan and Bosnic, 1994). Therefore, a logical and effective step used by many industries is sedimentation, which usually reduces SS, BOD and COD, thus decreasing the cost of effluent disposal. A

different retention time during sedimentation produces effluents of varying degrees of clarity and slurries or sludges (Alloway and Ayres, 1997).

Industrial wastes, and sludges in particular, do not appear to receive attention until they are deemed a public nuisance. Industry is then expected to find an alternative disposal method, usually quickly, *and* to convince people that this method is acceptable. Unfortunately, many industries consider waste disposal as something that erodes profit and are reluctant to invest in research, especially independent research, into the biological nature of the wastes they produce. In addition, essentially any research that is carried out tends to be kept within the industry due to commercial sensitivity. Hence when a problem does occur, there is often little independent scientific data available from which well-informed decision can be made (Alloway and Ayres, 1997).

This thesis deals with two industrial sludges; one from woolscouring and one from fellmongering. The aim was to describe their decomposition under different conditions and determine their potential effect on the soil microbial biomass (MB).

1.2 THE WOOL SCOURING AND FELLMONGERING PROCESSES

Sheep are dual-purpose animals raised in New Zealand for their meat and wool, and in the 1994/95 season, New Zealand had 49 million sheep (Britland, 1995). The sheep industry contributed \$2,297 million to New Zealand exports in 1995, equivalent to 35.4 % of export earnings by the main agricultural produce (Statistics New Zealand, 1997). Wool production alone contributed \$1,253 million (19.3 % of agricultural export earnings). The sheep industry comprises several smaller industries involved in the preparation of wool and skins for further processing, e.g. the scouring of wool and fellmongering of skins. New Zealand wool and leather industries are under increasing pressure to provide processed goods that include clean wool and tanned leather. While the economic side of value-added-products is good for New Zealand industries, environmental damage may occur if indiscriminant waste disposal occurs.

1.2.1 THE WOOL SCOURING PROCESS

To transform raw (greasy) wool into a form suitable for yarn production, the wool must be washed; this washing is called woolscouring. The scouring of wool involves firstly, "opening and dusting" of wool; the wool is emptied into a machine that teases the clumps of wool apart, shaking the wool vigorously all the time. This step removes much of the loose material and the opened wool then enters the washing process. The wool receives three hot water washes with non-ionic detergents, two cold rinses and a final hot rinse, after which the wool is dried with hot air and re-baled (Figure 1.1) (Stewart, 1983).

In 1994 there were 34 woolscouring plants in New Zealand (Wool Record, 1994; Britland, 1995)¹, most of which were associated with rural communities; 16 of the scouring plants were located in towns of less than 20,000 people (Crawshaw, 1990). Each town supplies water and electricity, and disposes of wastes for the town and the wool scour. An average wool scouring plant will produce post-sedimentation effluent equivalent to a town of 20,000 to 30,000 people (Robinson, 1981; Rodmell and Wilkie, 1983; Robinson, 1991). With over 80 % of New Zealand's annual clip scoured within the country (personal communication from WRONZ), this translates into the fleeces of essentially 49 million sheep (plus slipe wool, which is the wool recovered during the fellmongering process of sheep skins) being scoured at relatively few locations. In the 1996/97 season 275,000 tonnes of greasy wool were produced in New Zealand, which was equivalent to 203,000 tonnes of clean wool, indicating that 72,000 tonnes of contaminants (on a dry weight basis) required disposal at 14 sites¹ (Britland, 1997).

A shorn fleece contains wool, woolwax, suint, dirt and moisture (see later for a description of these materials), the amount of each varying considerably between sheep breeds and geographic location (Stewart, 1983). Woolwax, suint and dirt are considered, by the wool industry, to be contaminants, and their removal is the objective of woolscouring.

Woolwax is produced by the sebaceous glands of sheep and continuously deposited on emerging wool fibres. There is a technical difference between woolwax

¹ A personal communication from WRONZ stated that in July 1998 there were 14 woolscouring plants in New Zealand, 4 of which were located in towns of <20,000 people.

and woolgrease. Woolwax is the greasy material associated with the fleece, whether on the sheep or on the shorn wool. Woolgrease (Table 1.1) is the greasy material associated with woolscour liquor and is the greasy material removed from the fleece.

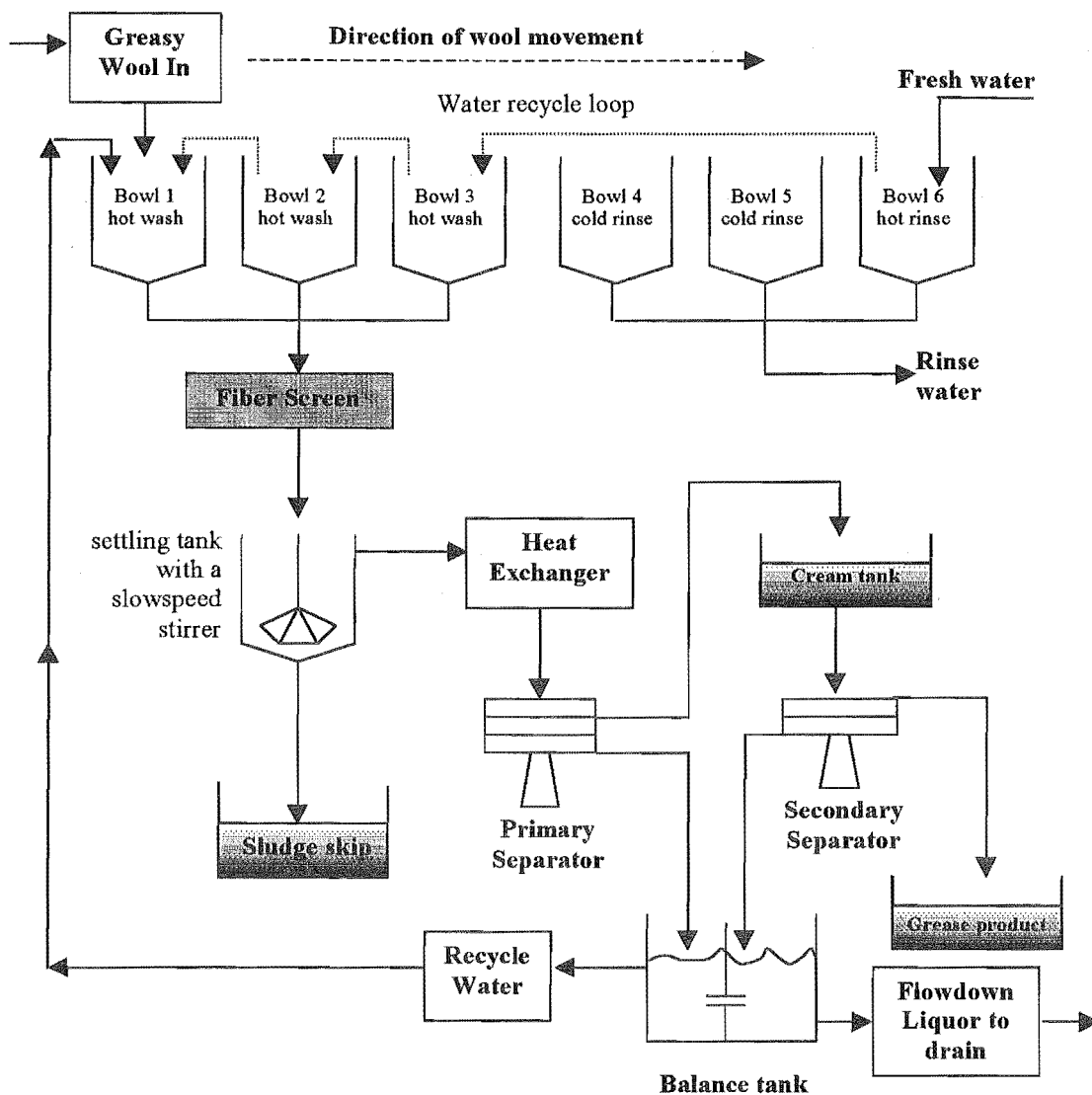
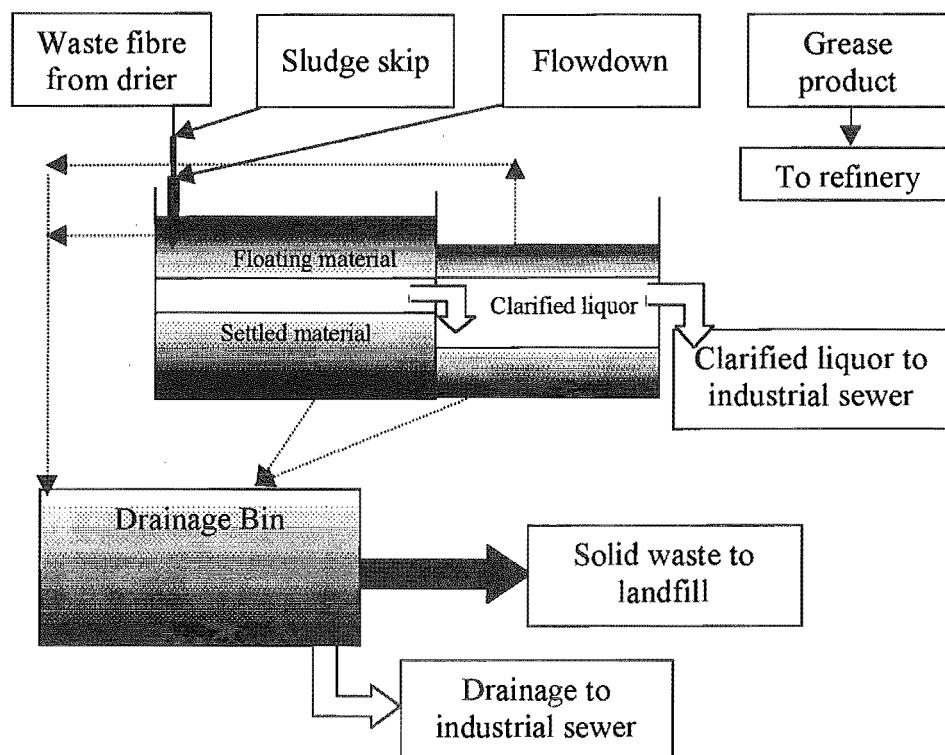


Figure 1.1. A generalised wool scouring train (WRONZ mini bowl scouring system).

Analysis of woolgrease indicates that it is composed of high molecular weight esters and does not contain glycerol. The acids of woolgrease esters may be straight or branched, with or without hydroxylation and range from C_7 to C_{41} . To indicate the complexity of woolgrease for microbial decomposition, more than 138 lanolin-acids

have been recovered after hydrolysis of lanolin, and lanolin represents only a small portion of woolgrease (Truter, 1956; Stewart, 1983; Barnett, 1986). The alcohols range from C_{12} to C_{36} and include aliphatic alcohols and sterols. In addition, the alcohols and



(Figure 1.1 continued)

acids present in woolgrease contain many bi-functional groups. Therefore, woolgrease is a substrate of considerable complexity for microbial degradation and contributes significantly to the COD and BOD of woolscour effluents (Stewart, 1983; Barnett, 1986).

Suint is the “sweat” of sheep and is the principal water-soluble fraction of greasy wool, and contributes significantly to the BOD of scour effluent (Table 1.1). Suint is composed mainly of potassium salts of organic acids (other cations include sodium, iron, aluminium, calcium, magnesium). Suint from New Zealand crossbred sheep tends to be alkaline, with a pH between 6.9 and 10.0 (Stewart, 1983).

Dirt, in the present context, is essentially soil and vegetable material trapped by the fleece, but it also includes faecal matter (Table 1.1). The dirt is usually finely divided material, typical of wind blown dust, which adheres to wool while the sheep are in the field. The dirt mixes heterogeneously with woolwax on the sheep, and contributes significantly to the SS of woolscour effluent (Stewart, 1983).

Table 1.1. The principal contaminants of greasy wool.

	Percent of contaminant on greasy wool ¹		
	Maximum	Minimum	Average
Merino Wool			
Grease	25.4	10.0	16.1
Suint	12.0	2.0	6.1
Dirt	43.8	6.3	19.6
Crossbred Wool			
Grease	8.5	1.6	5.2
Suint	12.1	2.2	8.0
Dirt + suint moisture	—	—	7.9

¹ Adapted from (Stewart, 1983)

Together, these three contaminants (woolgrease, suint and dirt) form an uneven layer over the wool fibres and the purpose of woolscouring is to remove as much of this layer as possible. The cost of effluent disposal, into municipal sewerage systems, is based on SS, BOD and COD (Stewart, 1983; Brach *et al.*, 1990; Buljan and Bosnic, 1994), thus a sedimentation step is essential when dealing with woolscour wastewater. For woolscour effluents the disposal cost is based on a hierarchy of BOD/COD > SS > volume for cost determination (Stewart, 1983). An average woolscouring train (2 m wide) will produce effluent with a COD ranging from 50,000 to 150,000 mg L⁻¹, BOD of 20,000 to 40,000 mg L⁻¹ and will contain 8,000 to 16,000 mg L⁻¹ woolgrease (Campbell *et al.*, 1980; Rodmell and Wilkie, 1983). This is equivalent to a scour train cleaning 1,500 kg of dirty wool per hour, and producing effluent containing 140 kg woolgrease, 145 kg dirt and 70 kg suint (per hour) (Bateup *et al.*, 1996).

A clipped fleece has 5 to 25 % woolwax (Table 1.1), of which all but 1-2 % is removed during scouring, with woolgrease contributing to about 16 % of the COD and about 40 % of the BOD of woolscour effluent (Stewart, 1983). The woolgrease fractionates into oxidised (major portion of woolgrease) and unoxidised grease (Stewart,

1983). The unoxidised grease is thought to come from the base of the wool staple (closest to the skin) and is almost totally recovered from the scour liquor by centrifuging the waste stream; the oxidised portion remains in the flowdown and thus becomes part of the sludge. In well-run scour trains as much as 25 to 40 % of the woolgrease may be recovered from the flowdown, in practice however, usually less than 10 % is removed (Stewart, 1983).

Water is extensively recycled during normal scouring for water and heat conservation. The recycled water enters a continuous flow centrifuge, which produces three fractions; a cream of unoxidised grease (cleanest grease fraction), a solids fraction of dirt and grease, and clarified water (the latter re-enters the cycle at scouring bowl-1, Figure 1.1). The effluent that leaves the scour train as spent liquor (called flowdown) has had 60-65 % of the dirt, 8-10 % of the grease and 10-15 % of the BOD removed after passage through the continuous flow centrifuge (Stewart, 1983).

The effluent is considerably "cleaner" after centrifugation and most research into handling woolscour liquors is aimed at reducing the level of contaminants in flowdown. The flowdown enters settling ponds, and it is there that much of the remaining grease forms a floating surface layer, while other materials settle out. The flowdown also contains wool fibre, which tend to float and associate with the woolgrease. The solids removed from the flowdown by centrifugation and the materials that settle from the flowdown form a settled sludge. Settled woolscour sludge will contain wool, soil, vegetable matter, woolgrease, suint, faeces, pesticides residues, and detergents. Woolscour sludge composition may vary considerably depending on the type of wool being scoured and effluent treatment available, which is often dictated by the resources of the local region. Often the sludge is allowed to accumulate for weeks or months before being disposed of in landfill, by incineration or by land spreading.

The different sludges (settled, balance tank, sediment, floating) can be removed as separate waste streams at any point during the waste cycle (Figure 1.1). Thus several waste streams are possible in the form of "sludge" and it is necessary to specify how the sludge was produced, for different methods will produce sludges of vastly different quantity and quality. Clarified effluent and rinse waters are disposed of either separately, together, and/or mixed with sludge(s). Therefore, different woolscouring operations may produce very different waste streams depending on how they combine

wastes. The level of grease and associated contaminants, such as heavy metals and pesticide residues, will vary between the different sludges produced.

Most of the pesticides used to control skin and wool parasites are lipid soluble and thus would be expected to become concentrated in the woolgrease (Greer, 1997). Pesticides that are used in New Zealand on sheep include cypermethrin, chlorfenvinphos, propetamphos, diazinon, coumaphos, dichlofenthion, deltamethrin, cyhalothrin, chlorpyrifos, cyromazine, and diflubenzron (Greer, 1997). It is reasonable to expect that New Zealand woolscour and fellmongery sludges will contain some of these pesticides and their residues (Johnson, 1990; Shaw, 1990; Baskaran *et al.*, 1996; Russell, 1996; Anon., 1997; Greer, 1997).

1.2.2 THE FELLMONGERING PROCESS

The first step in leather manufacture is the fellmongering process, which involves hair removal from freshly slaughtered animal skins followed by pickling (Figure 1.2). Wool removed during fellmongering is called *slipe wool*, which is recovered and scoured.

Animal skin is composed of three layers: the thin outer layer called the epidermis through which the hair and wool grow, the thicker middle layer called the dermis (or corium) in which the hair follicle is located, and the inner subcutaneous fatty layer. Alteration of these layers is required before leather can be produced.

Raw skins, which need to begin the fellmongery process within a few hours of sheep slaughter, are washed in large rotating drums with cold water. This step removes most of the *suint* (see section 1.2.1), which means that wool recovered from the fellmongery process requires 50 % more detergent for scouring due to the loss of natural saponification properties of *suint* (Stewart, 1983). The wastewater from the initial washing step is generally discharged directly to an effluent drain (industrial sewer if available) with little, if any, treatment (drain #1 in Figure 1.2). The skins have excess water spun out in a centrifuge; the wastewater from this also discharges into a drain.

The *flesher* scrapes off the subcutaneous fatty layer, immediately after the skins have been spun. The *flesher* produces a solid waste stream that is usually landfilled. Once the flesh side of the skin has been scraped, the skin is attached to a template, flesh

side up, and excess skin is trimmed from each skin, so that all skins are of standard size and shape. This trimming stage also produces solid waste, which may also be landfilled or used as a component in compost production. Some fellmongeries recover the wool from these skin pieces by natural rotting of the skin. Wool recovered in this manner (called pie wool or slipemaster wool) is premium slipe wool; it has not been in any contact with depilatory (Stewart, 1983).

After trimming, and while the skin is still attached to the template, the flesh side of the skin is coated with depilatory. The depilatory most commonly used is a lime-sulfide paste, which diffuses through the skin and dissolves the wool follicle, allowing the wool to be pulled out after 3 to 24 hours. The lime sulfide removes fat and soluble proteins, as well as making the hair follicle proteins soluble. The skins are stacked painted sides together until ready to have the wool mechanically pulled from them. The skins are removed from the templates and manually fed into the wool puller.

From the wool-pulling step two processing lines now emerge: the wool and the dehaired skin. The wool is dried, sorted and baled for transport to a wool scourer. On arrival at a wool scour the slipe wool requires opening and dusting (see Section 1.2.1), to remove the ends of the wool that are matted and eroded from depilatory contamination (Stewart, 1983). Slipe wool represents about 50 % of the profit for most fellmongeries that process primarily sheepskins.

A fleshed and dehaired skin is called a pelt. The pelt enters a series of rotating drums to neutralise the depilatory. Several different treatments are used, usually mild acids (such as boric, acetic or lactic acids) or acid salts (such as ammonium chloride, ammonium sulfate or sodium bisulfite) to neutralise the alkali. After neutralising, the pelt is "bated". Bating enzymatically removes interfibrillary proteins, which makes the pelts soft and pliable. The wastewater from these steps enters a separate drain (drain #2 in Figure 1.2), which feeds into an oxidation tank. In the oxidation tank manganese is added to catalyse the oxidation of sulfide to sulfate. The oxidised wastewater then joins with the wastewater from drain #3 (Figure 1.2) and feeds into a dissolved air flocculation tank. The pelt is washed in cold water and pickled. Pickling is usually done with a salt (e.g. NH_4Cl) and sulfuric acid, pesticide treatments may be added at this step. The wastewater from pickling/pesticide treatment goes into a drain (drain #3

in Figure 1.2), as does the drainage from the pelts after pickling. The final fellmongering steps of bating and pickling produce a clean white pelt ready for tanning.

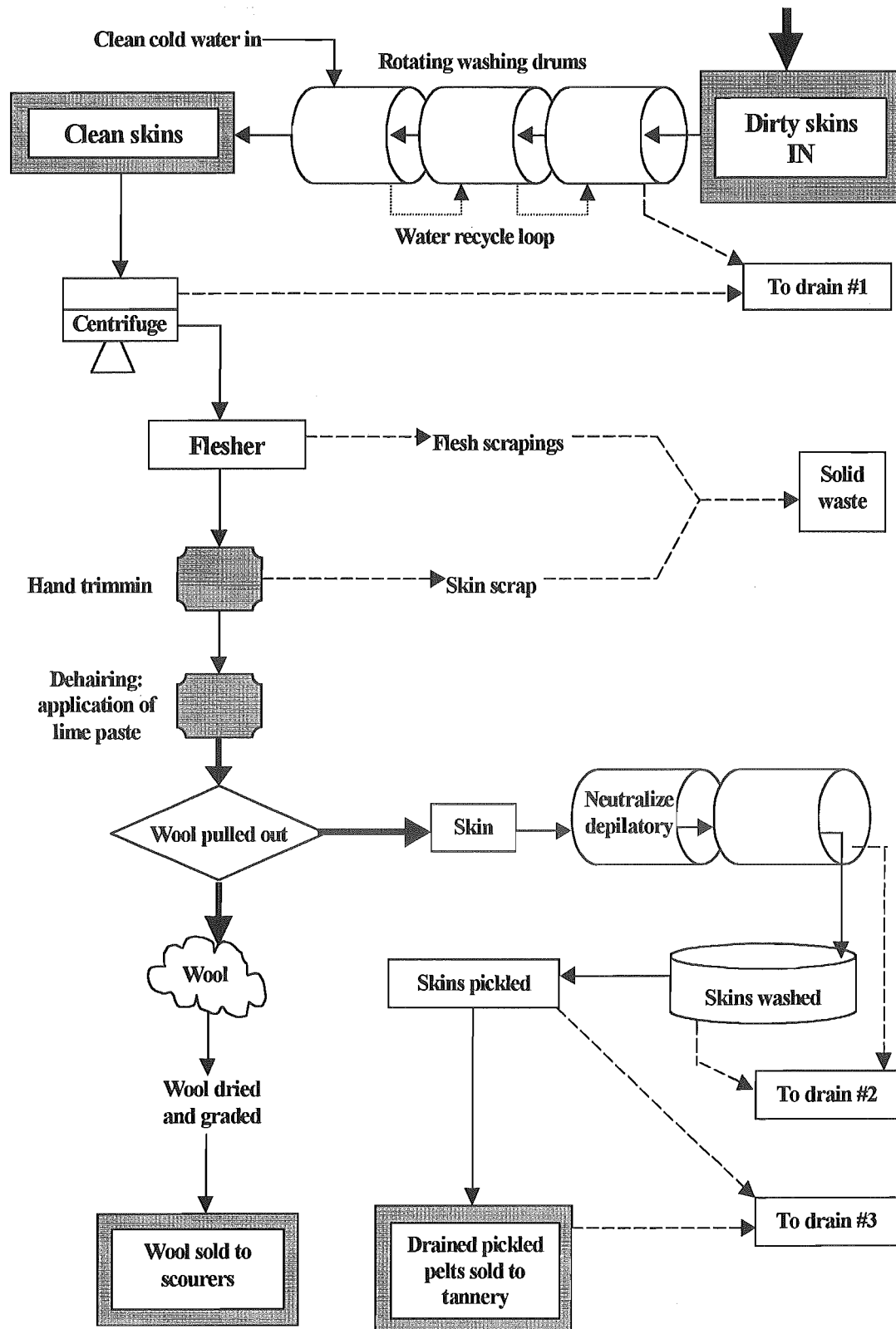
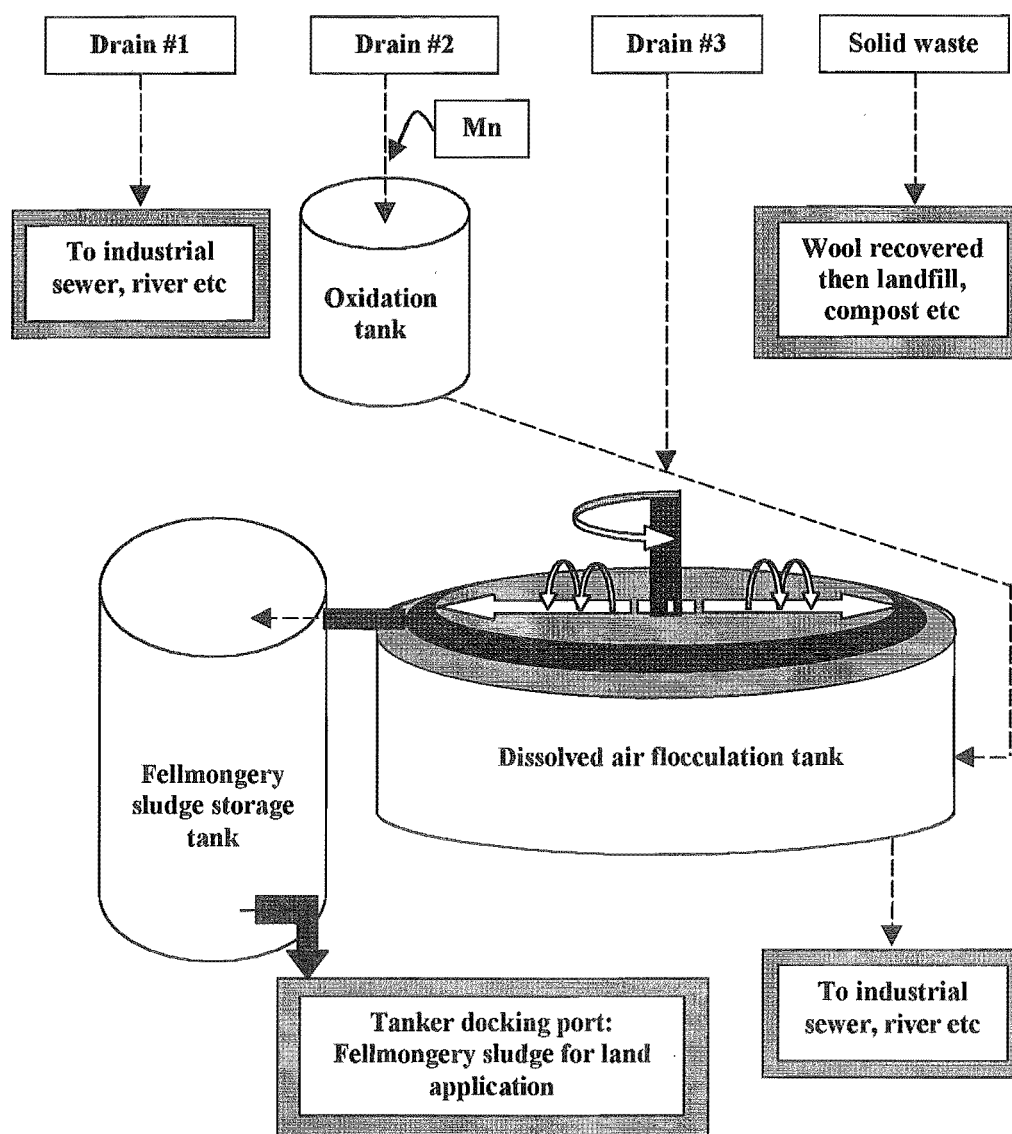


Figure 1.2. A generalised fellmongering process. Key: Skin movement ———→
Waste generated - - - -> Recycled water ·····→



(Figure 1.2. Continued)

Like woolscouring, leather manufacture is another agriculturally based industry with a high pollution potential (Talinli, 1994). A plant processing 2000 sheepskins per day could be expected to produce 90 to 120 cubic metres of wastewater per day. Typically, the wastewater has an average COD of 8000 mg L^{-1} (6760 mg L^{-1} as soluble COD), BOD of 3900 mg L^{-1} , total SS of 1090 mg L^{-1} , total N of 90 mg L^{-1} and 100 mg L^{-1} chromium III (Talinli, 1994). Additionally, effluents tend to contain quantities of hair and flesh, which adds to the general discoloration and malodorous nature of the wastewater (Sheikh and Irshad, 1980). Some reduction in the pollution loading of

wastewater has been achieved by the Sirolime process (where hides are impregnated with hydrosulfide before the liming step), and produces an effluent with a COD of 2000 mg L⁻¹ and SS of 240 mg L⁻¹ (Creagh, 1991).

The wastewater produced during fellmongery is too high in COD, BOD and SS for direct disposal into waterways or into industrial sewers. As in the case with woolscouring, during the primary clarification step a sludge is formed. In one clean-up process, the wastewater (e.g. from drain #3 in Figure 1.2) enters a dissolved air flocculation tank, with the wastewater entering at the bottom of the tank, and dissolved air carries a scum of material to the top. Electrolyte solution is gently sprayed over the wastewater's surface, which aids flocculation of the sludge. A rotating arm, of a screw shape and motion, moves the scum off the tank's surface and in to a pipe, which carries it to storage tanks ready for collection by waste disposal contractors. This scum is fellmongery sludge (Figure 1.2). There are no published figures on the nature of New Zealand fellmongery sludge, therefore the general characteristics of fellmongery sludge produced in the Mazamet region of southwestern France are given in Table 1.2.

Table 1.2. General chemical characteristics of fellmongery sludges¹ produced in the Mazamet region of France (from (Plat *et al.*, 1984)).

Constituent	% dry matter
Organic matter	55.1
Carbon	42.7
Total Nitrogen	3.6
NH ₄ ⁺ -N	1.55
Fats	25
Proteins	22.5
Ash	44.9
Total Phosphorus	0.16
Total Potassium	0.49
Total Calcium	Traces
Total Magnesium	0.09

¹ The pH of the sludge was 4.3, the water content was 68.7 %, and the C-to-N ratio was 11.9.

1.3 DISPOSAL OF ORGANIC WASTES

Rubbish dumps were a fact of early civilisations, and have been a source of disease and pollution ever since, although recent management of solid wastes is reducing this problem in developed countries. Domestic animals, such as pigs, were allowed to eat raw sewage and garbage, often passing diseases onto humans in their meat and faeces. Certainly disposal of human effluent was a major problem for early cities, with most dumped into the streets to be washed away by periodic floods. Many wealthier people chose to live directly by waterways so their “privy” could discharge to the river (Anon., 1986).

Many methods have been used to dispose of industrial waste. Early methods were direct discharge into local waterways, in the same way human effluent was discharged. The advantage of this mode of disposal was that the mess was immediately removed from sight, and so began the mentality of “out-of-sight, out-of-mind”. Most industries intentionally sited themselves by rivers, and as industrialisation and urbanisation proceeded these rivers received an admixture of human and industrial wastes. For example, in Bradford, England, the River Aire has received effluent from wool scouring since 1311 (Anon., 1986). In the 1700s, the river would spontaneously catch fire, with grease from the woolscouring effluent thought to be the principal fuel for these fires (Truter, 1956).

1.3.1 COMPOSTING OF WASTES

It is probable that composting of organic wastes such as food scraps and human effluent was amongst the early attempts to deal with waste, although the emphasis may not have been directed towards improved crop yield. Composting is a microbiological process that has gained renewed popularity recently as a worthwhile and rational method to reduce solid organic waste. The initial impetus came from municipal sewage works, usually because current methods of sludge disposal were becoming too expensive or the methods, such as dumping sludge at sea, were being banned under international agreements. The use of composted material as fertiliser and soil conditioner fulfils the ideals of recycling nutrients and sustainable management of wastes (Del Mazo Suarez *et al.*, 1987; Anderson, 1990; He *et al.*, 1995; Alloway and Ayres, 1997; Illmermer and Schinner, 1997).

During the early phase of composting, microbes utilise easily decomposable materials and their increased metabolism causes the internal temperature of a compost heap to rise above ambient, reaching 60 to 70 °C (Godden *et al.*, 1983; Hirai *et al.*, 1983; Morel *et al.*, 1985; Sikora and Sowers, 1985). Under these new physical conditions some microbes cannot function; their place is taken by a new range of microbes that are either tolerant of high temperature or, as is more usual, are thermophilic by nature. The residues of dead organisms contribute to the compost heap, thus giving the new-phase organisms readily metabolisable substrates. Eventually, the abundance of readily decomposable C and N falls and the metabolic rate of the microorganisms decreases, and a corresponding decline in temperature occurs, which is mirrored in the alteration of the microbial component from thermophilic to mesophilic. The final phase of composting is curing, which allows the slow transformation of organic matter into a stable state that can then be incorporated into soil without causing adverse effects (Hirai *et al.*, 1983; Morel *et al.*, 1985; Anderson, 1990). Put succinctly, the aim of composting is to biotransform organic waste material into innocuous useful material.

The determination of compost maturity is difficult, and using compost that is not completely composted is not advisable. Immature compost has a higher N requirement than the soil into which it is applied, consequently N immobilisation rather than N release is likely to occur (Hirai *et al.*, 1983; Morel *et al.*, 1985; Anderson, 1990). Associated with this, if the compost is too young there will be a high oxygen demand by microorganisms as the compost continues to decompose, which has the potential to deprive plant roots of oxygen. Thus, both these aspects of immature compost will have a negative effect on plant growth (Anderson, 1990).

Compost is intended to act as soil organic matter; increasing water holding capacity, aggregation, cation exchange capacity, aeration and soil structure. The diversity of microbes is usually increased when composted material is incorporated into soil (Atlas, 1988). Microbial diversity in the rhizosphere can afford plant roots a degree of protection from attack by pathogenic organisms, due to microbial competition for resources (Atlas, 1988).

Compost is not an alternative to fertiliser in agricultural land use; it may complement and act as a slow release fertiliser, providing a balance of trace nutrients and minerals (Kirchmann, 1990; Inbar *et al.*, 1993; Mahimairaja *et al.*, 1994). Through its

nutrient balance and soil-improving nature compost allows added fertilisers to be better utilised by plants, and usually, less fertiliser is required to achieve similar crop yields when compost is incorporated into the soil (Epstein *et al.*, 1978; Castellanos and Pratt, 1981). The improvement comes mainly from the microbial biomass (MB) being in a more amenable environment and therefore able to utilise incoming nutrients more efficiently, then release them back to the plants as the growing season advances.

1.3.2 THE USE OF FERTILISERS ON AGRICULTURAL LAND

For hundreds of years people have been amending soils in order to achieve greater crop production. Initially fertilisers were organic in nature and comprised farm-derived waste. From about 1860 the use of inorganic fertilisers began; usually N, P and K were added in this form. In parts of Europe from about the 1880s, the use of inorganic fertilisers significantly increased crop yields, and the increase was in proportion to the application rate of the fertiliser (Finck, 1982). Unfortunately, there can be a high degree of wastage and loss of fertiliser nutrients post-application due to nutrient sorption, leaching and volatilisation if the application of fertiliser is not co-ordinated crop requirements. Often specific nutrients, such as N, are not available to plants in the quantity needed at the time they are needed, e.g. during seed development (Wild, 1988).

Healthy plant growth is achieved when a constant supply of nutrients is available, rather than periodic excesses, which tends to occur with inorganic fertilisers. Consequently, consistency of nutrient supply, more than quantity, is often a key feature for maximum plant production (Wild, 1988). The build-up of the soil MB acts as a sink for incoming nutrients. The MB can then act as a slow release fertiliser, providing a constant supply of nutrients as the microbes die off (Harris, 1988 a, b, c).

The addition of excess fertiliser, in order to meet the minimum level of the most limiting nutrient, a common practice in the past, is not justified. The actual utilisation of applied fertilisers by plants is often low, as shown by examples in Table 1.3, the remainder may be leached or immobilised within the soil matrix (Finck, 1982). For a long time soil in most countries has coped well with some degree of indiscriminate amendment. Now, however, scientific rational is being used to achieve high productivity without compromising the integrity of soil systems.

Table 1.3. Nutrient utilisation from applied fertiliser after the first year of application. From (Finck, 1982).

Fertiliser	Utilisation Rate (%)
Nitrogen, mineral	50 – 60 ¹
Nitrogen, organic	20 - 30
Phosphate, mineral	15
Phosphate, organic	20 - 30
Potassium (mineral or organic)	50 - 60
Manganese, Copper, Zinc (mineral)	0.5 - 5

¹ Studies using N¹⁵ have indicated that utilisation rate may be 30-40 % (examiner's comments).

The organic matter level of a soil can be used as a general indicator of soil health, and an increase in organic matter is generally associated with improved soil fertility and water holding capacity (Haynes and Williams, 1992; Elliott, 1997). However, it must be kept in mind that “bigger is not always better”, meaning that there are situations where high organic matter and its build up may not, necessarily, indicate a healthy soil. For example, peat bogs are not regarded as productive soils yet they have high organic matter (Swift *et al.*, 1979).

The turnover of organic matter is generally considered to be of greater importance than the gross level of organic matter. There are situations where a soil should be classified as healthy, even though the low biodiversity may not suit the description of “healthy soil” (Sparling, 1997). For instance, soil in early successional stages and in extreme environments, such as sand dunes, and alpine and polar regions, are examples where biodiversity and fertility are typically low, but the soil should be regarded as healthy. Soils at the ecosystem climax, such as tropical rainforests which have high biodiversity but low fertility, are still healthy (Sparling, 1997). Therefore, the intended use of soil to which organic sludges (such as sewage, woolsour, and fellmongery sludges) may be applied will help determine which indicators of health are suitable and reasonable to measure.

1.3.3 LAND-BASED DISPOSAL OF WOOLSCOUR AND FELLMONGERY SLUDGES

Sludges from woolscouring and fellmongering represent only some of the waste streams originating from these industries and therefore only a fraction of the overall

waste associated with the sheep industry (Stewart, 1983). If sludges are to be disposed onto neighbouring land or into small landfill sites associated with towns, it is important that sludge composition (quantity and quality) be known. Without this knowledge the local ecosystem may sustain damage from which it cannot easily recover. This damage may not be obvious until remediation becomes extremely costly, if even possible.

For example, the disposal of woolscour and slaughterhouse effluents on to a Waimakarari stony silt soil had dramatic effects on the vegetation (Figure 1.3 through to Figure 1.8)² and raises questions about the effect that this form of waste disposal may have on the soil. In Figure 1.3 the pasture had been sprayed with both slaughterhouse and woolscour effluent three months previously (information on the actual application rates were not available). Walking through the paddock, the region that had received the slaughterhouse effluent (far left in the photograph) was about knee-deep in grass. The lushness of the grass suggested that the slaughterhouse effluent had been beneficial, however, this appearance was deceptive. Sheep had recently grazed the paddock, but there were *no* sheep droppings on the slaughterhouse effluent-sprayed section of the paddock, suggesting that the sheep had not grazed this section.

The central strip of Figure 1.3, which had not received any effluent, had been so heavily grazed by sheep that much of grass was gone from below the soil surface, indicating that the sheep had eaten roots and all. A similar grazing pattern was seen around the entire parameter of the paddock and 4 to 5 cm under the fence into neighbouring pasture. Hence, at first glance, the untreated strip of pasture appeared to have lower grass productivity than the treated strips of pasture; again the appearance was deceptive since the sheep seemed to prefer to eat the untreated grass.

² The woolscour effluent that was used on these fields was *not* the same woolscour sludge used for the experimental work reported in this thesis.



Figure 1.3. A grazed pasture on to which slaughter house effluent (far left strip, inside the fence), no effluent (central light strip) and woolscour effluent (green strip in the foreground) had been applied three months previously. Sheep had grazed the paddock and their droppings are the dark speckles on the central strip. See text for details.



Figure 1.4. A pasture that had received woolscour effluent (central brown strip) and slaughterhouse effluent (green strips left and right) two weeks previously.

The foreground strip in Figure 1.3 had been sprayed with woolscour effluent and appeared moderately lush with grass but contained relatively few sheep droppings. Together these observations suggested that the sheep had found the grass sprayed with slaughterhouse effluent unpalatable, thus it had grown tall, and they found the grass sprayed with woolscour effluent only marginally palatable. The sheep had preferentially grazed the untreated grass extremely heavily (central strip and margins), and as there was ample grass available, it suggested that the sheep may have been hungry for untainted grass. The quality of the wool produced during this period of time may have been compromised due to dietary stress.

Figure 1.4 shows another pasture that had received woolscour and slaughterhouse effluents only two weeks prior to when the photographs were taken. The woolscour effluent-treated grass was brown and shrivelled, whereas the grass that had received slaughterhouse effluent was green. A closer look at the soil surface that had received woolscour effluent (Figure 1.5) indicated that not all the grass was dead, although patches of the desiccated effluent formed an almost continuous layer over the plants and soil. To look even closer (Figure 1.6), the woolscour effluent had completely smothered a broad-leaf weed (a dandelion) and the shimmer of the greasy material over the soil surface can be seen (lower left of Figure 1.6).

Within this same paddock, and between the strips receiving slaughterhouse effluent and woolscour effluent, a gorse bush had been evenly sprayed with effluent on each side (Figure 1.7). The side hit by the slaughterhouse effluent appeared undamaged by the spray, whereas woolscour effluent had damaged the flowers and foliage. The woolscour effluent had drained off the bush and a considerable area at the base was thick with air-dried effluent, some patches up to 8 mm thick (Figure 1.8).

These observations indicated the immediate impact that the disposal of woolscour effluent had had on the vegetation, and one can speculate that the soil microbes and animals may have also been affected. However, the effect on the vegetation was temporary from a visual perspective, as seen in Figure 1.3, which had general re-growth of grass. There are sites around New Zealand that have received woolscour wastes for many years and there is no evidence that sheep have suffered undue effects from grazing at these sites (Campbell *et al.*, 1980). It does, however, place waste disposal in perspective; Figure 1.3 through to Figure 1.8 are illustrations solely of waste disposal.



Figure 1.5. A close-up photograph of pasture that had received woolscour effluent two weeks previously, with woolscour scum caking the surface. The scale is approximately half the actual size.



Figure 1.6. A close-up photograph of woolscour scum on a pasture that had received woolscour effluent two weeks previously. The photo is approximately to scale.



Figure 1.7. A gorse bush located between two strips of pasture that were sprayed with slaughterhouse effluent (side with yellow flowers) and woolscour effluent (side with brown foliage) two weeks previously.



Figure 1.8. The area at the base of the gorse bush, on the side sprayed with woolscour effluent, showing the effluent that had accumulated at the base of the bush.

The disposal of fellmongery sludge onto pasture did not have a dramatic effect on the vegetation and the paddocks visited were evenly grazed (Figure 1.9 and Figure 1.10)³. The sludge appeared to have a scum on the surface when only two days old, which was not apparent after seven days (Figure 1.10). Fellmongery sludge left a greasy layer over the grass, however this did not appear to limit grass growth.

Woolscour and fellmongery sludge generation, both in quality and quantity, is highly dependent on the processing plant involved (Stewart, 1983). No two plants are expected to produce exactly the same sludge, similarly no two sludges from the same operation are expected to be exactly the same. The variability comes from the actual feed stock entering the processing plant.

The wool type scoured has a large impact on the amount of sludge produced (e.g. dags tend to contribute a large amount of soil and manure to the waste) (Stewart, 1983). Similarly, the types and state of skins (e.g. how clean they are, whether there is much blood, fat and flesh adhering) received at a fellmongery affects the quality of the sludge. It is this inherent variability in sludge quality that means biological studies into nutrient cycling are required to calculate application rates of woolscour and fellmongery sludges that will promote sustainable disposal. However, no distinction is made regarding the chemical nature of the organic N, and applications for Resource Consents (under the Resource Management Act, 1991) to spread sludges on land do not include mineralisation studies (personal observation; Canterbury Regional Council, 1995).

In theory, land application of organic waste (e.g. sewage sludge) is based on mineral N uptake of crop/pasture, and is related to the initial mineral N present and how much of the organic N is expected to be mineralised during the growing season (Terry *et al.*, 1981). Therefore N mineralisation studies (which are completely lacking for woolscouring and fellmongery sludges) should indicate the potential for sludge organic N to be transformed to mineral N. Such a transformation rate can then be used to determine a suitable sludge application regime (Castellanos and Pratt, 1981; Vazquez-Montiel *et al.*, 1995).

³ The fellmongery sludge that was used on these fields was similar to that used for experimental work reported in this thesis, and was from the same fellmongery operation.



Figure 1.9. Pasture that had received fellmongery sludge two days previously, showing a scum on the surface.



Figure 1.10. Pasture that had received fellmongery sludge one week previously.

Consideration of other nutrients in the waste also need to be taken into account, as sometimes the application of organic wastes to land, based on N content, will over-supply other nutrients, particularly P. This is often a problem with disposal of poultry house bedding waste, which is high in both N and P (Brinson *et al.*, 1994; Kingery *et al.*, 1994; Nichols *et al.*, 1994; Mahimairaja *et al.*, 1995). A second consideration for land application of organic wastes and sludges is the potential toxicity of the sludge, at actual rates of application. This type of assessment involves determination of pathogenic organisms, heavy metals and pesticide residues. Finally the effects that sludge disposal may have on neighbouring landowners is considered before granting a permit (e.g. odour and visual problems; Canterbury Regional Council, 1994). The issuing of permits and subsequent monitoring of sludge disposal is the responsibility of the local and regional councils.

There does not appear to be any work published concerning the N dynamics of woolscour and fellmongery sludges. McAuliffe (1984) investigated both dairy factory and woolscour effluents, and found that woolscour effluent applied at a low rate of 10 to 30 mm caused a 28 % decline in soil hydraulic conductivity, and when applied at 50 mm caused a 50 % decline. This decline was attributed to physical clogging of soil with grease and suspended solids. Similar clogging occurred with the dairy factory effluent, but this was attributed to proliferation of microbial populations. The permeability of soil was found to recover after about 6 days, although recovery was highly temperature dependent. At 20 °C, soil recovered in 6 days, but at 10 °C it took more than 21 days before showing signs of recovery, which is consistent with microbial degradation of substrates within the effluents (McAuliffe, 1984).

The temperature in the top 10-cm of the soil profile will fluctuate on a daily and annual basis depending on the amount of energy received from the sun. In New Zealand (Hutt Valley) the average monthly soil temperature at 5 to 30 cm depth is typically below 20 °C all year (McLaren and Cameron, 1990). Therefore, woolscour effluent applied to New Zealand soil has a strong potential to cause a considerable reduction in soil drainage due to suspended solids and grease (e.g. Figure 1.6), which still may occur even if the effluent is applied in the warmer summer months. In some areas prone to drought the water that accompanies the effluent may be as valuable as the nutrients, and often wastewater and sludges are viewed as unbalanced fertilisers with irrigation potential (White, 1979).

Indirect evidence suggesting the decomposition of woollscour effluent comes from pilot studies aimed at cleaning up the flowdown (see Figure 1.1). These studies have reported large reductions in BOD and COD (Rodmell and Wilkie, 1983; Cail *et al.*, 1986; Lapsirikul *et al.*, 1994 a, b, c). Aerobic treatment of flowdown caused an 86 % reduction in BOD (from influent level of 471-g m⁻³ to effluent level of 57-g m⁻³), an 85 % reduction in grease, and 72 % reduction in COD (Rodmell and Wilkie, 1983). Similar clean-up efficiencies were found under anaerobic conditions when more than 56 % of the COD and 47 % of the grease were removed (Cail *et al.*, 1986).

Extreme caution should be exercised when interpreting results such as these. The treatment has obviously been successful in producing a “cleaner” effluent, however there was no evidence that mineralisation had occurred. The material that contributed to the elevated SS, BOD and COD may simply have *shifted phases* and now be part of the sludge, which is not usually analysed for enrichment (Rodmell and Wilkie, 1983; Cail *et al.*, 1986). For example, anaerobic bioflocculation treatment of flowdown caused a successful reduction in grease of about 85 %, and a reduction in COD of 60 to 90 %, which is impressive taken at face value (Lapsirikul *et al.*, 1994a). However in a second paper (Lapsirikul *et al.*, 1994c), it was reported that there was *no net change* in the grease level of clarified effluent *and* sludge, which suggested that the grease loading of the woollscour effluent had simply moved from the effluent into the sludge.

The biological dynamics of sludges produced during effluent treatment need to be investigated, and mineralisation studies (e.g. C or N) are essential. Woollscour and fellmongery sludges may be significantly enriched with grease, metals and pesticide residues as the pressure to produce cleaner effluent increases. With minimal monitoring of the actual nature of sludges, permits issued for sludge disposal *via* land application do not consider the changing nature of the sludge. A change in the chemical composition of the sludge may alter its decomposition rate and hence its decomposition could be faster if more readily mineralisable material is available, or slower if there is an increase in more recalcitrant substrates. For example, a permit to dispose of the fellmongery sludge used during research for this thesis on to pastoral land in Christchurch was issued in 1994 and expires in 2028 (Canterbury Regional Council, 1994).

1.4 NUTRIENT CYCLING

When sludges are applied to agricultural land it is assumed that they will break down and contribute nutrients to the crop. The release of nutrients from organic material is called nutrient cycling. Nutrient cycling is defined by the decomposability of a resource, under relevant incubation conditions and involves the cyclic conversions of nutrients from one form to another within biological communities⁴. Decomposition is in turn dependent on the quality of substrates with in the resource for microbial metabolism and conditions that influence microbial metabolism. Plant litter has been used extensively to describe substrate quality, decomposition and nutrient cycling.

1.4.1 PLANT LITTER

Plant litter, usually described as tissue that is no longer part of a living plant, is the largest input to the decomposer system (Swift *et al.*, 1979). Leaves form only 2 % to 10 % of the plant biomass in a forest, but they represent between 40 % and 70 % of the litter (by weight) (Swift *et al.*, 1979). Leaf senescence withdraws nutrients prior to abscission; therefore, the leaf litter reaches the ground in a state well altered from fresh leaves. The nutrients that remain are in a variety of forms, but most are organic molecules of different complexities. Generally plant litter can be considered to be made up of six categories: (i) cellulose, (ii) hemicellulose, (iii) lignin, (iv) water-soluble substances, (v) alcohols and other organic solvent-soluble substances, and (vi) proteins (Swift *et al.*, 1979). The structure and amount of these molecules influences the rate at which decomposition will occur. However, climatic conditions, especially temperature and moisture, tend to have the greatest effect on decomposition due to the influence on microbial metabolism (Swift *et al.*, 1979).

1.4.2 SUBSTRATE QUALITY

The rate at which leaf litter decomposes will be influenced strongly by the most limiting nutrient (to the decomposers) at a specific moment in time (Swift *et al.*, 1979). The C-to-N ratio of soil organisms is usually lower than the material they are decom-

⁴ Personal communication from Dr. E. G. Gregorich.

posing; suggesting that available N may limit the decomposition rate of most plant detritus. As decomposition proceeds the C-to-N ratio of detritus decreases as C is respired. The chemical nature of decomposing material also alters over time. For example proteins may form complexes with cations or humus, which causes some degree of protein precipitation (Swift *et al.*, 1979). Precipitated proteins tend to be less accessible to the mineralisation processes. Hence, while the C-to-N ratio of the resource may drop, the N remaining may not be any more bioavailable. Mineralisation of N is occurring at the same time as immobilisation of N, and a measurement indicates which process is dominant at that time. Usually when substrate C-to-N ratio is <30 then N will be mineralised (Harris, 1988c).

Resources used by microorganisms vary in composition. The C-to-N ratio of a resource is not always a strong indicator of the actual quality as a substrate, as not all the C and N is equally available (Swift *et al.*, 1979). For cellular uptake, molecules must be small enough to move through the microbial plasma membrane. This movement may be passive (diffusion) or active (specific transmembrane sites with ATP-requiring channels). Usually water-soluble molecules are the most available to microbes, as these molecules can move along diffusion gradients into the cell or they can be specifically taken up with other molecules (either passively or actively). Therefore, an initial indicator of substrate quality is the proportion of the material that is water-soluble. For most organic substrates, such as plant residues, very little is water-soluble; most is present in complex forms, usually polymeric in nature (Swift *et al.*, 1979). Hence, the polymers of nature ultimately dictate the "quality" of a resource. For microbes to obtain nutrients or energy from polymers, depolymerisation must occur. This catabolic step is inevitably achieved by extracellular enzymes, which may remain membrane-bound or be fully secreted into the surrounding medium. Enzymatic cleavage releases smaller units (usually monomeric), which can then be taken up by the organism.

One problem with the release of extracellular enzymes is whether the exoenzyme is active in the matrix into which it is secreted; the pH may not be optimum for enzyme activity or there may be substances present that are specifically inhibitory. Another problem encountered is, as a consequence of cleavage of the polymer, the organism that secreted the enzyme is not the only organism that can utilise the monomer; once cleaved the unit is available to other organisms within the immediate area. Therefore, secretion

of extracellular enzymes, while essential, may have a high energy cost if not all the cleaved units are utilised by the secreting organism. The next marker of substrate quality is what proportion of a substrate's polymers can be cleaved by either non-specific enzymes or by enzymes that are broadly expressed by the local microbial population (Swift *et al.*, 1979).

Once a polymer is cleaved, the unit removed may need modification before it can be transported into the cell. Usually this modification is one that increases water solubility (often by hydroxylation), sometimes however more specific modifications are required to enable molecules to pass through transport channels (Atlas, 1988). The third indicator of substrate quality is whether the cleavage enzymes facilitate an increase in the water-soluble nature of the unit or whether other enzymes are required for more complex alterations. Enzymes that are broadly and constitutively expressed for the conversion of a unit into a form that can be taken up by the cell, the higher will be the substrate quality (Swift *et al.*, 1979; Atlas, 1988).

Resources of different qualities produce residues of different types, some of which may be resistant to further degradation by a large proportion of the microbial community or even be toxic to the soil organisms (Swift *et al.*, 1979). If there is a residue, then there is a reason why decomposition stopped short of total mineralisation. The simplest explanation for mineralisation stopping is that the bond cleavage now requires specific enzymes, and a representatively smaller proportion of the microbial population expresses these enzymes. A residue, however, is also a resource that is available for decomposition (Figure 1.11).

It was hypothesised by Winogradsky in 1924 that two pools of microorganisms operate in soil, the zymogenous population and the autochthonous population (Jenkinson, 1966). This division of microbial activity is almost certainly an over simplification, but does help explain biological activity in soils. One pool is active only when new substrates are added to the soil and is termed zymogenic, this population rapidly increases as the new energy sources are exploited, then declines as the substrate becomes more and more specialised in the enzymes that are required to transform it. The zymogenic population spends much of its time in a dormant state and probably represents the largest proportion of the MB. This pool of microorganisms will mineralise some of the added organic matter and transform much of the remainder (Swift *et al.*, 1979). The second pool of microorganisms is active most of the time, but

at low levels and is the autochthonous population. This population is not strongly influenced by incoming raw material, and probably cannot rapidly increase its population size. Many of its members may have the burden of plasmids, which have been implicated in many of biochemical pathways for degradation of complex molecules (Atlas, 1988). Microbes carrying plasmids may not be as competitive against the genetically simpler microbes for easily metabolised substrates (Watson *et al.*, 1988). It is thought that the autochthonous pool is responsible for the slow and steady turnover of soil organic matter.

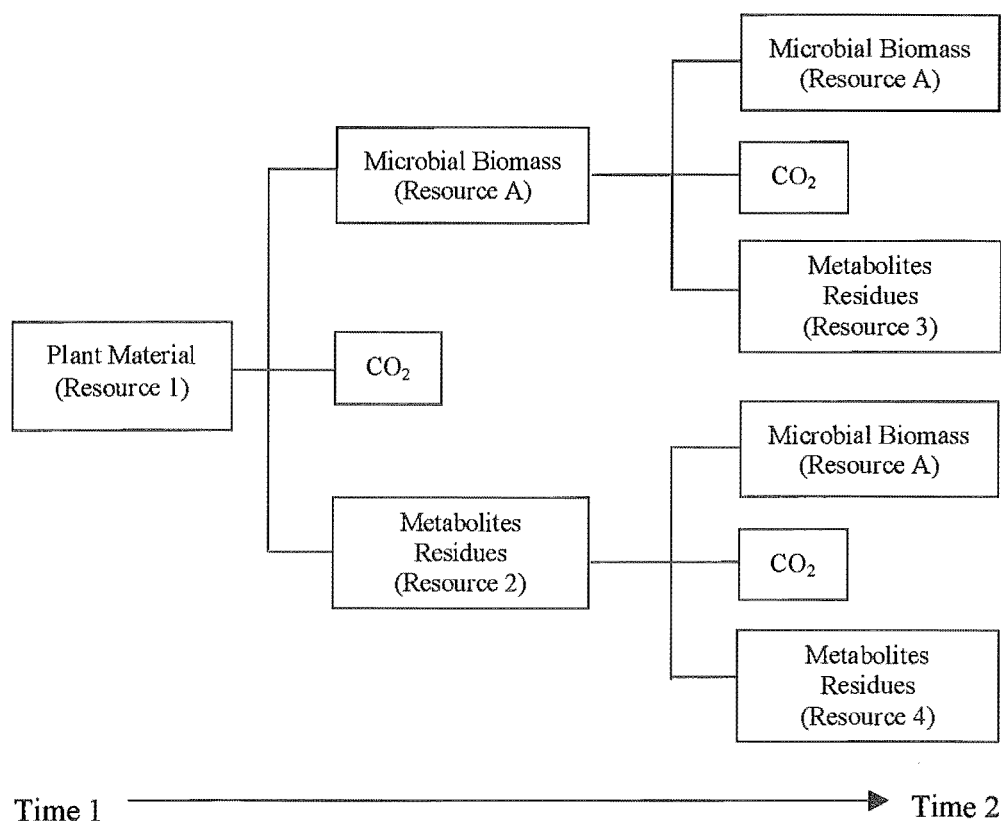


Figure 1.11. The decomposition of plant material and the turnover of the microbial biomass, over a period of time. Modified from (Swift *et al.*, 1979; Jenkinson and Ladd, 1981)

Thus, before any monomers (or small units) can be formed, the more complex bonds must be altered or broken. When these bonds are broken (or altered) then the modified substrate acts as fresh organic matter, and is again utilised by most of the zymogenous microbial population (Harris, 1988a). Hence, the microbial community

moves from substrate to substrate always using the material with the most readily available C. Materials such as manure or organic sludges will require many rounds of exposure to quite specific groups of microbes before appreciable mineralisation has occurred (Atlas, 1988). Therefore, over time organic matter contains more residues that require specialised enzymes for their degradation.

In summary, while the C-to-N ratio of a resource will give a crude indication of substrate quality, it is the fraction of the substrate that is water-soluble and the fraction that resists depolymerisation that determines substrate quality. Overall, the proportion of a substrate with complex bonds (or complex stereochemistry) finally dictates the overall substrate quality. This is of course a simplistic rendition of substrate quality. Other factors (climate, soil pH and moisture) influence microbial activity, but not how decomposable a substrate is.

1.4.3 DECOMPOSITION

Decomposition of organic matter is the alteration of a resource by biotic and abiotic processes, over a period of time, controlled by the simultaneous interaction of leaching, catabolism and comminution (Swift *et al.*, 1979). Decomposition implies significantly more alteration to a substrate than processes such as methylation or hydroxylation, which can imply that a compound has "decomposed", when it has only "disappeared" as determined by gas chromatography. Decomposition releases fragments than can be mineralised, which is the highest degree of decomposition, and can be illustrated using plant litter as a model (Figure 1.11). Wooldscour and fellmongery sludges would be expected to conform to nutrient cycling models, however this has not been tested.

Figure 1.11 indicates that while the initial resource changes over time, the microbial component remains similar, i.e. their C-to-N ratio remains relatively steady. Fungi have a cellular C-to-N ratio of about 15:1 and bacteria about 5:1. If a resource contributes 100 units of C, and a fungus can utilise 50 of these units, then to maintain its cellular C:N ratio of 15:1 a fungus requires 3.3 units of N. Thus, the resource needs to have 100 C: 3.3 N (C-to-N ratio of 30) (Killham, 1994). If the resource has more N than required (thus a lower C-to-N ratio, say 20:1) then net mineralisation of N will occur; the fungus releases N in the process of obtaining C from the resource. However,

if the resource has less N than required (a higher C-to-N ratio, say 60:1) then N must be taken up from the immediate environment to meet the N demand for maintenance of the cellular C-to-N ratio. Hence net immobilisation of N will occur. Thus the recycling of nutrients from different resources permits the MB to remain steady as an important resource.

Loss of substrate weight over time has been used as a reliable method by which to assess the decomposition of organic matter, the weight loss being due to evolution of volatile metabolites, e.g. CO₂ (Swift *et al.*, 1979). Mineralisation studies are perhaps the most widely used methods to determine decomposition (Terry *et al.*, 1979; Beare *et al.*, 1992; Jensen, 1994). Nitrogen mineralisation indicates the potential availability of nutrients in organic matter and is usually assessed using microcosm experiments under different physical conditions (Bremner, 1965b; Keeney, 1982). Insufficient N is a key factor limiting optimum plant growth (Wild, 1988), thus the rate at which microorganisms can mineralise organic N is of great importance to primary productivity. In most soils the N is locked up in organic forms and can usually only be released by microbial action, which is strongly influenced by climatic and soil conditions (Wild, 1988).

1.4.4 NUTRIENT CYCLING

Nutrient cycling is the replenishment of soil nutrients in an available form that permits transfer of nutrients between different trophic levels. The transformation of N is an excellent example of this (Figure 1.12; Swift *et al.*, 1979). This transfer is relevant whether within an ecosystem or between ecosystems. It is essential that nutrients be constantly cycled for at least two reasons. Firstly, without cycling, plants would cease to produce new biomass and food chains would collapse. Secondly, plant and animal debris must be recycled or a vast accumulation in organic matter would occur. Peat bogs are a good but specialised example of very slow decomposition (Swift *et al.*, 1979).

Soil is a component of ecosystems, and is built up by the interaction of plants, animals and microbes. Most plants and microbes can exist independently of each other, however, both tend to grow better when in the presence of the other. The biological fraction of soil is determined by both physical and biological parameters. Plants,

microbes and fauna, and their individual or collective processes, strongly influence the nature of soil, with the existence of one potentially determining the existence of the other. Plants also influence nutrient cycling, other than their litter fall, by addition of simple organic substances to the rhizosphere by root secretion. This is thought to stimulate the microbial population by providing substrates for energy production; there is a high-energy demand for the decomposition of the more complex organic matter fractions (Swift *et al.*, 1979). Recently fixed photosynthate is thought to be released into the rhizosphere, where it is immobilised by microbes or respired by roots (Meharg and Killham, 1988).

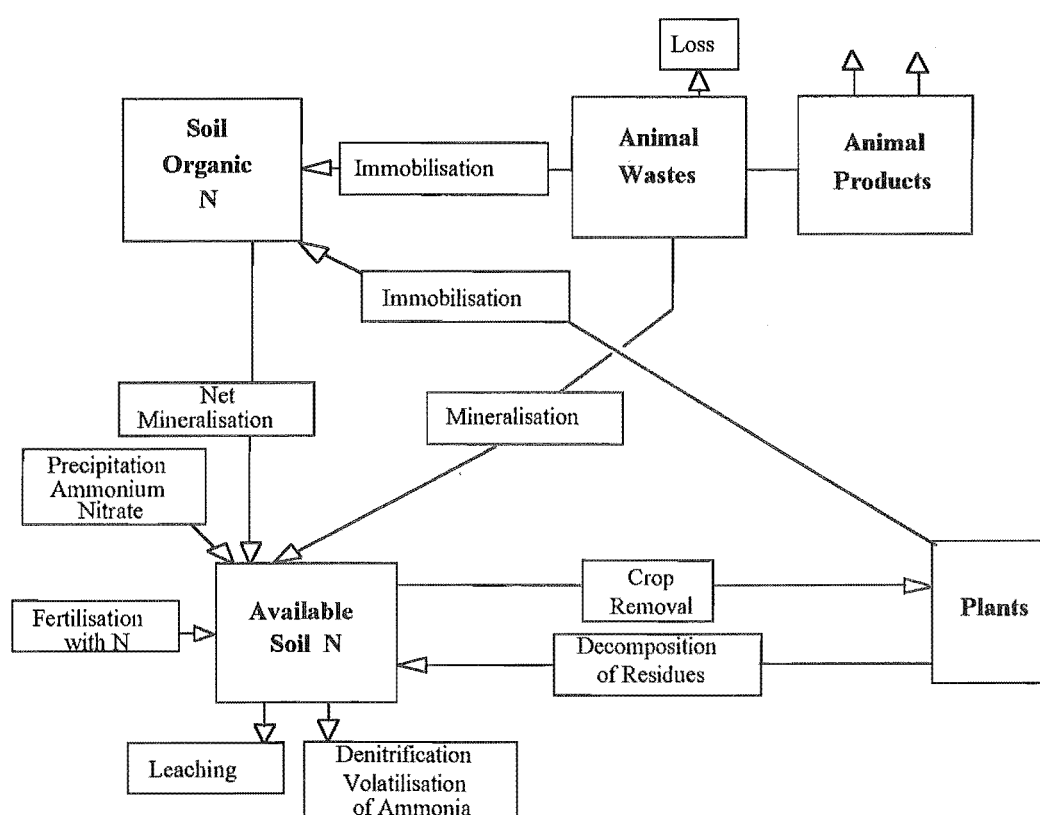


Figure 1.12. Model of N cycling during the decomposition of an organic substrate (from Swift *et al.*, 1979).

Many factors influence the primary colonisation of detritus, and, from a microbial perspective, size is important. Bacteria tend to be dominant in the decomposition of small-size particles, whereas fungi are usually the primary colonisers of larger, tougher material (Swift *et al.*, 1979). The animals that feed on plant litter significantly contribute to the decomposition process by progressively reducing the fragment size

until it becomes small enough for microorganisms to complete the nutrient cycling and mineralise the material. While plant material is in the digestive system of soil fauna, microorganisms associated with the litter are usually in a better environment for their enzymes to utilise complex organic matter. Thus, when the faeces are passed, the mineral nutrient content is often higher, and the vegetative microbial population increased (Swift *et al.*, 1979).

The microbes themselves are a source of food for many soil organisms. Nutrients immobilised within the soil MB are released by the grazing of MB by microvores (Swift *et al.*, 1979; Ingham *et al.*, 1985; Powlson, 1994). The exponential function used to describe microbial growth is probably better thought of as a successional pattern of growth from the zymogenic microbes represented; rather than all zymogenic organisms responding as "one". One group increases in number first, and another group closely follows as the first group starts to die, using the first group as an easily utilised source of energy to facilitate degradation of the more difficult fractions of organic matter. The autochthonous microbes will also use the dead zymogenic organisms as an energy (and nutrient) source, however their metabolic rate is much lower, and may truly reflect the historical availability of carbon (Bradley and Fyles, 1995). Therefore, decomposition and nutrient cycling are both diverse and dynamic. Mineralisation is much more restricted than decomposition and reflects the excess of nutrients to the decomposer organisms, especially the microorganisms.

Microbial C is generally two orders of magnitude lower than the total C of soil (Bradley and Fyles, 1995). In soil, microorganisms obtain their energy from the catabolism of organic matter. Soil organic matter may be complex, with relatively insoluble substrates such as polyphenols, tannins and lignin, or chemically simpler, with molecules like starch, cellulose, soluble proteins and DNA. Microbes utilise the nutrients liberated from organic matter for cell maintenance and, if sufficient energy/nutrients are available, cause an increase in MB (Killham, 1985). As most of the substances are rich in C compared to other nutrients, carbon dioxide is the main mineralisation product from microbial metabolism.

Generally, the simpler organic molecules formed during decomposition are substrates for a new suite of microbes, which may possess a slightly different enzyme complement, allowing a differential attack on components. Thus, nutrient cycling is

decomposition working on micro-scales and controlled by substrate quality and climatic conditions (Figure 1.12).

1.4.5 MICROBIAL BIOMASS

The MB is defined as the living component of soil organic matter, excluding macrofauna and plant roots (Jenkinson and Ladd, 1981). Microorganisms immobilise nutrients and increase in biomass. Microvorous organisms then release the nutrients by grazing on the MB (Swift *et al.*, 1979; Ingham *et al.*, 1985; Meharg and Killham, 1988; Powlson, 1994). Bacterial numbers and the amount of active mycelium increase, but hyphal length does not, due to grazing by microvores. When the MB is grazed by microvores, microbial C is respired and N is released for cellular growth and maintenance (Ingham *et al.*, 1985; Bradley and Fyles, 1995). The net result appears to be an increase in microbial populations when they are grazed rather than a decrease, and this is thought to be due to the enhanced cycling of nutrients (Ingham *et al.*, 1985).

Therefore, it appears that nutrients from the rapid turnover of MB are important to the fertility of soil and plant growth. It is the labile fraction of soil organic matter that has a large impact on the rate of nutrient cycling and on the amount of any specific element released. Nitrogen is usually a limiting nutrient for plants, and its release from organic substrates is a convenient parameter for substrate quality determination (Figure 1.12).

Unfortunately, the MB is easier to define than measure. Many methods for determination of MB have been investigated over the last 50 years and most books on soil biology include a comprehensive range of techniques with their advantages and limitations (Weaver *et al.*, 1994; Schinner *et al.*, 1996). For example, plate-count methods indicate organisms culturable only on the media provided and greatly favour organisms that have rapid growth or those that reproduce by sporulation. Thus, the organisms cultured do not, necessarily, reflect their proportion of the microbial population (Atlas, 1988). Direct counting of soil microbes by microscopy, including the use of stains to distinguish living from dead, is hindered by the small size of most soil organisms, their clumping habit and the opaqueness of soil particles, although it does permit the assessment of morphology (Frederick, 1965). Indirect, physiological methods include substrate induced respiration, use of biological markers, such as ATP

and muramic acid, and fumigation methods. The indirect methods are standardised against known quantities of bacteria and fungi, however they are still only semi-quantitative and best interpreted as "a numerical qualification" rather than quantification.

In current physiological methods, minimal emphasis is placed on identification of individual organisms, rather the ability of the microbial population to immobilise C and N (fumigation-extraction method) and respond to added substrates (substrate induced respiration method) is considered important. Thus, the function of the MB as a whole is assessed by these latter methods that estimate MB.

The fumigation-extraction (FE) method for estimation of MB involves the partial sterilisation of soil with chloroform (CHCl_3), and subsequent extraction of the TN made soluble by CHCl_3 (Jenkinson and Ladd, 1981; Brookes *et al.*, 1985b; Vance *et al.*, 1987; Ross, 1989). The principle is that CHCl_3 disrupts cytoplasmic membranes of soil organisms, making the cytoplasmic contents extractable. The difference between a sample fumigated and a sample not fumigated represents the N that was previously contained within the cells (flush-N). Different research groups have proposed conversion factors (k_{EN}) that translate flush-N into an amount of MB; k_{EN} is usually based on experiments using pure cultures of fungi and bacteria added to soil and their specific recovery determined. The FE method is used in this thesis to estimate the MB of sludge-amended soils.

1.5 SEWAGE SLUDGE

There is a large difference between quality of organic matter from plants (primary resources) and animals (secondary resources, which includes woolscour and fellmongery sludges). Plants tend to invest their C in cell wall structures (polysaccharides), whereas animals (and microbes to a lesser extent) tend to incorporate more C in lipids and proteins (Swift *et al.*, 1979). Therefore, while the decomposition of plant litter provides a conceptual model, it is not the ideal substrate with which to compare the decomposition of woolscour and fellmongery sludges; a better substrate may be sewage sludge. Over the last 30 years sewage sludge has received considerable research attention, and during the early studies basic decomposition studies were used to

provide biological data that could then be incorporated with chemical data when determining sludge application rates. Similar studies are used in this thesis to describe the decomposition of woolscour and fellmongery sludges.

1.5.1 SEWAGE SLUDGE BIOLOGY AND CHEMISTRY

Animal manures have long been utilised as a source of available nutrients, and are usually returned to arable land as fertiliser. The use of the human equivalent, night soil or sewage wastewater, on vegetable plots was probably a compromise between adding fertiliser and disposal of waste when people settled in one place for a period of time. Urbanisation has led to a large amount of sewage requiring disposal over a relatively small area; it is not the nature of the sewage that is a problem, rather it is its quantity.

Sewage enters a processing plant, is filtered (the solid material is one fraction of the sewage sludge), and usually undergoes at least one biological digestion phase, which produces a solid fraction that is added to the sewage sludge. Finally the wastewater is filtered again before the clarified effluent is discharged to water bodies such as a river or the sea. The resulting sludges are applied to land, landfilled or incinerated (Davis, 1988; Gay, 1988; Garvey *et al.*, 1993; Spinosa *et al.*, 1994; Walsh, 1995).

The material entering the sewage plant may contain industrial as well as domestic wastes. Sewage wastewater, and the sludges in particular, usually contains elevated levels of metals and other compounds that have not traditionally been applied to agricultural land (McGrath *et al.*, 1995). As processing of sewage wastewater is refined, the effluent is "cleaner", but greater quantities of sludge are produced and these sludges potentially have more contaminants such as metals and pathogens, making sludge disposal more difficult.

In the United Kingdom, 30 % of the sewage sludge produced has been disposed of at sea, however in 1998 this practice will be prevented under international treaties (McGrath *et al.*, 1995). Almost half the total sewage sludge produced is returned to agricultural land, but only about 10 % of the arable land is used for sludge disposal. Therefore, for Great Britain, as more sludge must be applied to land, there is a greater risk that metals and pathogenic organisms may contaminate land.

A description of various sewage sludges is presented in Table 1.4. These figures indicate that the sludges contribute significant quantities of N and P, making them valuable as fertilisers. However a quick survey of the metals associated with them indicates potential environmental problems (Wheatley, 1988).

Table 1.4. Description of some sewage sludges for nutrients and heavy metals.

Variable	Authors and sludge type				
	Mitchell	Mitchell	Terry	Brendecke	Chander
	<i>et al.</i> , 1978	<i>et al.</i> , 1978	<i>et al.</i> , 1979	<i>et al.</i> , 1993	<i>et al.</i> , 1995
	Aerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic
	%				
Ash	36	61	64	—	—
Total C	—	—	26.3	25.2	48.5
Total N	4.2	0.6	1.8	4.4	2.43
Nitrate	0.014	0.014	—	0.021	—
Total P	1.71	0.96	1.4	0.82	—
Available P	0.75	0.46	—	—	—
	ppm				
Cu	1700	900	—	568	401
Zn	1100	3400	—	800	1354
K	7400	4600	—	—	—
Cd	14	105	—	7	8
Cr	200	1200	—	32	—
Pb	400	700	—	89	—
Ni	124	214	—	26	154

When sewage sludges are applied to land, metal contaminants are likely to affect nutrient cycling (Epstein *et al.*, 1976; Terry *et al.*, 1979). An increased level of metals in soil, plants, microbes and fauna has been found at almost all sites where sewage sludge has been applied to land (Brewer and Barrett, 1995; Chander *et al.*, 1995; Weissenhorn *et al.*, 1995). More than 90 % of the metals applied to agricultural land in sewage sludges remained in the top 15-cm of soil (McGrath *et al.*, 1995). Even when

the last application of sewage sludge was more than 25 years previously, more than 70 % of the applied metals were present in the top 20 cm of soil (McGrath *et al.*, 1995).

Metals tend to adhere strongly to soil organic matter and clay, and at normal soil pH ranges (6 to 8) they are relatively immobile and generally not available to plants, but they may affect enzymatic processes (Viets and Boawn, 1965; McLaren and Cameron, 1990). For example N-fixing cyanobacteria are sensitive to metals, with about a 50 % reduction in N fixation in soils treated with sewage sludge (McGrath *et al.*, 1995). While a subsequent reduction in plant growth, especially of red and white clovers, following application of sewage sludges may be due to metal toxicity on the plant, it is more likely to be due to the effect that the metals have had on N-fixing bacteria (Chopp *et al.*, 1982).

1.5.2 SUBSTRATE QUALITY AND DECOMPOSITION OF SEWAGE SLUDGE

The quality of sewage sludge can be assessed by decomposition studies using C and N mineralisation and the effects of the sludges on the MB and soil fauna. When added to soil, synthetic anaerobically-digested sewage sludges respired 20 to 45 % of the added C, suggesting that a significant fraction of sewage sludge remains in the soil (Terry *et al.*, 1979).

The N dynamics of sewage sludges indicated, that firstly, aerobically digested sludge mineralised 51 % of their initial N whereas anaerobically digested sludge mineralised only 28 % of added N (Mitchell *et al.*, 1978). This suggests that the anaerobic treatment had caused a significant alteration in the substrate quality and reduced the amount of labile organic-N, principally by denitrification. Secondly, similar proportions of the applied N were mineralised irrespective of the level of sludge application, suggesting a similar fraction of labile organic material (Epstein *et al.*, 1978). Thirdly, that sewage sludge has the potential to undergo extensive nitrification during decomposition, with 27 to 37 % of the applied N mineralised as nitrate (Epstein *et al.*, 1978; Mitchell *et al.*, 1978; Terry *et al.*, 1981).

The application of sewage sludge to soil increased the level of metals present, especially copper and zinc (Mitchell *et al.*, 1978; Chang and Broadbent, 1982; Brookes *et al.*, 1986; McGrath *et al.*, 1995). Usually soil contaminated with metals, from applied sewage sludge showed a decline in C and N mineralisation compared with soil that had

received non-contaminated farmyard manure (Epstein *et al.*, 1976; Epstein *et al.*, 1978; Mitchell *et al.*, 1978; Sommers *et al.*, 1979; Terry *et al.*, 1979). The MB was found to be increased (by 30 %) when arable soil was amended with sewage sludge contaminated with low or high levels of metals (Fliebbach *et al.*, 1994; Chander *et al.*, 1995). However, when results are presented as a ratio of $C_{\text{microbial}}$ -to- C_{organic} the metal-enriched sludges significantly depressed the ratio (Fliebbach *et al.*, 1994), suggesting significant stress on the MB (Killham, 1985).

The population structure of soil fauna reflected enhanced microbial growth at low sludge application, with increases in earthworms, bacterial-feeding nematodes, and nematodes that feed on nematodes (Mitchell *et al.*, 1978). The lack of increase in fungal-feeding nematodes may reflect that fungi do not respond as well as bacteria to land application with sewage sludge. This could be a pH effect as many sewage sludges tend to be neutral to alkaline in nature (Terry *et al.*, 1981; Armon *et al.*, 1994; Bierman and Rosen, 1994; Sawhney *et al.*, 1994). However, at higher application rates, nematodes that grazed on fungi were significantly decreased in amended soils, as were spiders, aphids and adult diptera (Mitchell *et al.*, 1978).

In summary, sewage sludge decomposes relatively slowly in soil. At low application rates, for sludges low in metal contamination, sewage sludge appeared beneficial to the MB, which suggested that nutrient cycling was not negatively affected. However, at higher application rates, and especially if the sludges are contaminated with high levels of metals, there was a negative effect on the soil MB, which is likely to depress nutrient cycling and lead to the accumulation of organic matter (Swift *et al.*, 1979).

1.6 WOOLSCOUR AND FELLMONGERY SLUDGES

Based upon the decomposition of plant litter in soil, models of nutrient cycling indicate the transfer of nutrients from one trophic level to another, via the microbial decomposer system (Figure 1.11 and Figure 1.12). At each passage through a cascade of biotic and abiotic functions and constraints, the original resource is altered into a new resource. The constraints upon the transfer, include factors such as temperature, moisture, atmosphere and matrix, are what regulate nutrient cycling. It is the effect of

these factors on substrate mineralisation that need to be ascertained when studying the decomposition of organic waste materials and the effect of the wastes on the soil organisms.

A typical problem for many agriculturally based industries is that the waste products exceed the disposal capacity of the production region. Usually the problem is one of concentration rather than innate waste toxicity, and the cost of waste disposal is, usually, a significant proportion of company profits. Yet with many industrial wastes there are fractions that it seems, paradoxically, a "waste" to "throw away". Such wastes may be rich in N, the lack of which commonly limits plant growth. The amount of N that potentially becomes available to plants is determined by the extent of waste mineralisation. Along with the degree of waste mineralisation, an understanding of the consequences of industrial waste decomposition, such as increased mobility of heavy metals or nitrate pollution of water, is also required. Maximising decomposition and promoting nutrient cycling from organic industrial wastes may involve changing the plant species usually cultivated and/or using species that tolerate the waste and mitigate potential pollution problems.

The timing of waste application to agricultural land needs to be carefully considered so that the nutrients are applied when the plants need them most, e.g. a cereal crop requires more N during seed-set than earlier in the growth cycle. The practice of applying inorganic fertiliser "on-mass", based on the premise that the minimum plant requirements will be met, is not such a common practice now. The availability of nutrients from the organic wastes when applied in this manner comes back to the extent to which constituents are mineralised and so enter the nutrient cycle. Therefore, a practical approach is required to determine the potential availability of organic waste nutrients to plants. At the same time, it is necessary to appreciate that only partial mineralisation that occurs over the usual time periods used for decomposition studies. There may be negative effects of the decomposition that need to be considered in conjunction with the positive benefits.

Woolscour and fellmongery sludges are relevant examples of wastes that could be beneficial to soil since they tend to be rich in N (Brach *et al.*, 1990; Christoe, 1996). However, there is insufficient information currently available to make well-informed decisions as to their suitability for sustainable land disposal. The mass processing of wool and animal skins has been conducted for more than 700 years, but we do not see

centuries-worth accumulation of these specific wastes. Therefore, it seems reasonable to suggest that the decomposition of woolscouring and fellmongering wastes would be consistent with the models proposed for nutrient cycling (Figure 1.11 and Figure 1.12). Data detailing C and N mineralisation under different conditions would indicate what proportion of woolscour and fellmongery wastes would be readily degradable and potentially available to plants, however this information is not available.

The wastewater research emphasis for the woolscouring and fellmongering industries has been to produce cleaner effluents that can be discharged directly into waterways or sewers (Stewart, 1983). The woolscouring industry has been generally successful in reducing the BOD and COD of scour effluent (McLachlan *et al.*, 1973; Smith and McLachlan, 1974; Chisnell and Robinson, 1979; Chisnell and Stewart, 1979; Gibson *et al.*, 1979). Published studies are not available for fellmongery effluents, however it is assumed, in a manner similar to woolscouring effluents, that significant improvement in effluent clarity would have been achieved over time.

Minimal research has focused on the material removed from the woolscouring and fellmongering wastewater during effluent clarification. Each time the effluent is treated to remove SS, BOD and COD the material removed becomes part of another waste stream, usually sludge. It therefore appears that the composition of sludges from woolscouring and fellmongering have the potential to change significantly, over time, as new technology is used to produce even cleaner effluent. If these changes are occurring then it is probable that the biodegradability of the sludges may alter with time; sludges may contain more material that is readily decomposed or they may progressively resist decomposition.

There are many different wastes that come from the woolscouring and fellmongering industries. The wastes are highly dependent on the quantity, quality and type of wool/skins entering the processing train, and on the type of treatment that the waste streams receive. Wastes produced from one operation will vary on a daily, weekly and monthly basis, and the wastes from one industry will vary to a greater extent due to in-house treatments used. It is therefore misleading to consider that information about one woolscour sludge or one fellmongery sludge will be applicable to all woolscouring sludges and all fellmongery sludges. With essentially no information that describes woolscour and fellmongery sludge decomposition, it is necessary to begin

data collection on a small scale. In this manner, methodologies develop that are relevant to other wastes from these industries.

In this thesis, simple methodology, similar to that used in the early studies for sewage sludge decomposition, was applied to determine the decomposition of woollscour and fellmongery sludges and their influence on soil organisms (consequences of decomposition). This thesis is not a survey of woollscour and fellmongery sludges. It is accepted that, had different sludges from these industries been selected for study, different results, showing broad variation around the means, would most likely have been found. By using only one sludge from each industry, a base level of decomposition can be determined, against which the decomposition of other woollscour and fellmongery wastes may be compared.

The woollscour sludge used in this work was collected from a Christchurch woollscouring operation, however it was supplied on the condition that the company would not be named in this thesis. The sludge was a product of three waste streams: (i) heavy solids from the first three scour bowls, (ii) settled solids and floating material retained during clarification of the flow-down liquor, and (iii) fibre and dust discharged from the wool dryers (dampened to prevent it blowing away). The heavy solids are periodically flushed into two settling ponds. The flowdown liquor (wash and rinse waters) enters settling ponds, and has a variable retention time of days to weeks, depending on the quantity of wool being scoured. Clarified liquor flows from a grating into a discharge canal, while settled and floating material is retained behind a grating. The sludge (settled and floating material from all three waste streams) is periodically removed from the settling ponds by a mechanical dredge and placed in a drainage bin. After several weeks of drainage the sludge is disposed of into landfill. As landfill is the final destination of this sludge there is no requirement for a resource consent for its disposal. Therefore, the woollscour sludge used during this research represents a waste stream that is essentially unmonitored in both quantity and quality.

The fellmongery sludge was collected from the Belfast plant of Colyer Watson Fellmongery (north of Christchurch). It was produced by dissolved-air flocculation and represented a cream skimmed from the surface of the balance tank (refer to Figure 1.2). The usual mode of sludge disposal is on to agricultural land (Figure 1.9 and Figure 1.10). The soil of these paddocks is a Selwyn sandy loam and has a water holding capacity of 40-60 mm (Canterbury Regional Council, 1994). The permit to discharge

fellmongery sludge onto 342 ha of grazed pasture was issued under section 105 of the Resource Management Act and included the following restrictions (Canterbury Regional Council, 1994):

- The level of Cr^{3+} in the sludge would not exceed 25 mg m^{-2} of pasture per year,
- The annual volume of sludge disposed onto the land would not exceed 12,000 cubic metres,
- The daily disposal would not exceed 90 cubic metres,
- A maximum depth of applied sludge would not exceed 4 mm per year, which was representative of one pass of the tanker per year,
- No sludge disposal is permitted within 20 m of any boundary, water race or creek,
- The permit's expiry date is 30 April 2028 (Canterbury Regional Council, 1995).

The common factor between the woolscour sludge and the fellmongery sludge used during this research was their sheep industry origin. However, the sludges were disposed of using different methods; the woolscour sludge went to landfill and the fellmongery sludge was spread onto agricultural land. The different methods of disposal raised the question whether these waste management options were the best ways to deal with the two sludges.

The sustainable management of waste materials has a general philosophy to “reduce, reuse and recycle” whenever possible. It could be argued that the disposal of fellmongery sludge onto agricultural land is a way of recycling sludge nutrients, i.e. a fertiliser. Therefore, a criterion used to determine how much sludge to apply to agricultural land (whether fellmongery or sewage sludges) is how much N the sludge contains. However, the potential benefits of using fellmongery sludge as a fertiliser may be off set by pollution problems, such as enrichment of soil with heavy metals and pesticide residues or the pollution of waterways with nitrate. The resource consent for fellmongery sludge disposal had no data that indicated the degree of decomposition expected when the sludge is applied to land, therefore the application of fellmongery sludge to land was solely waste disposal. Therefore, an essential question to be answered for the disposal of fellmongery sludge is whether it has potential as a fertiliser.

The woolscour sludge used during this work is usually disposed of in landfill, which is not considered a sustainable method of waste disposal (Alloway and Ayres,

1997). Specifically, landfill does not permit the sludge nutrients to be recycled for crop production and secondly, the decomposition conditions in a landfill are primarily anaerobic, which reduces the energy produced during sludge decomposition. If the woolscour sludge were to be disposed of on agricultural land, in order to reuse/recycle nutrients, the level of N in the sludge would be a principal determinant of how much sludge to apply.

Therefore, for both the woolscour and fellmongery sludges the aim of the thesis was to assess their decomposition. This aim was addressed by determining: (i) the degree to which the sludges decompose, (ii) the constraints on their decomposition, and (iii) the consequences of sludge decomposition.

2 MATERIALS AND METHODS

2.1 HYPOTHESES TESTED AND EXPERIMENTAL DESIGN

The basic question to be answered during this research was whether the woolscour and fellmongery sludges could be used as fertilisers. When organic wastes are used as fertilisers an application rate is selected that usually reflects the N requirements of the crop. Hence, a crude assumption is made that the N within each waste is equally mineralisable. An assumption such as this is testable. Thus, the first two hypotheses tested for the decomposition of woolscour and fellmongery sludges were H1 that the sludges would show similar levels of decomposition, and H2 that the sludges would mineralise similar amounts of N under different conditions (Table 2.5).

Table 2.5. Hypotheses addressed during the study of woolscour and fellmongery sludges.

H1	The woolscour sludge and the fellmongery sludge will decompose to a similar extent under controlled conditions of temperature and moisture.
H2	The woolscour sludge and the fellmongery sludge will mineralise similar amounts of N under similar environmental conditions.
H3	The presence of woolscour sludge or the fellmongery sludge will be not alter the amount of net-N mineralisation during decomposition of other organic substrates.
H4	Woolgrease will not inhibit the decomposition of protein.
H5	There is no change in net-N mineralisation during the decomposition of the woolscour sludge amended with readily available nutrients.
H6	Less than 1 % of applied N will be leached as nitrate during any single leaching event during the decomposition of fellmongery sludge.
H7	Similar amounts of plant biomass will be produced from a matrix amended with fellmongery sludge as from an unamended matrix.
H8	Similar MB will be present in a matrix amended with woolscour or fellmongery sludges as in an unamended matrix.
H9	The MB of woolscour and fellmongery sludge-amended and unamended soils will respond in a similar manner when soil is incubated under ambient conditions.

The results gained when testing these first two hypotheses permitted the formation of new hypotheses. Altogether nine hypotheses were tested during this research, and while they are all presented above, the hypotheses were progressively determined (Table 2.5). The experimental set up that was used to test the hypotheses is in Table 2.6 below. The relationship between different hypotheses and the results is presented in a flow chat in the Results Section (Figure 3.15).

Table 2.6. Experiments used to test the hypotheses regarding the decomposition of woollscour and fellmongery sludges.

Hypothesis	Experimental
H1 Sludge decomposition over time	a) Weight loss during decomposition (with incubation at 22 °C and 40 % moisture). b) C mineralisation by trapping evolved carbon dioxide (in a sand matrix with incubation at 22 °C and 20 % moisture). c) N mineralisation by trapping evolved ammonia and KCl-extractable ammonium and nitrate/nitrite (in a sand matrix with incubation at 22 °C and 20 % moisture).
H2 N mineralisation under different conditions	a) Effect of temperature on N mineralisation (in a sand matrix with incubation at 20 % moisture for 50 days). b) Effect of moisture on N mineralisation (in a sand matrix with incubation at 22 °C for 53 days). c) Effect of anaerobic conditions on N mineralisation (in a sand matrix with incubation at 22 °C and 30 % moisture for 108 days). d) Effect of sand and soil on N mineralisation (with incubation at 22 °C and 20 % moisture for 30 and 54 days).
H3 Effect of sludges on nutrient cycling	a) N mineralisation when the woollscour sludge was co-incubated with the fellmongery sludge (in a sand matrix with incubation at 22 °C and 20 % moisture for 50 days). b) N mineralisation when the woollscour sludge or the fellmongery was co-incubated with flower petals (in a soil matrix with incubation at 22 °C and 20 % moisture for 50 days). c) N mineralisation when wool (raw and scoured) and the woollscour sludge were decomposed in soil previously amended with woollscour sludge (in a soil matrix with incubation at 22 °C and 20 % moisture for 30 days). d) N mineralisation when the fellmongery sludge was decomposed in soil previously amended with fellmongery sludge (in a soil matrix with incubation at 22 °C and 20 % moisture for 30 days).
H4 Woolgrease effect on protein decomposition	a) N mineralisation from casein when mixed with different amounts of woolgrease (with incubation at 22 °C and 20 % moisture for 116 days).

<p>H5</p> <p>Decomposition of woolscour sludge (available nutrients)</p>	<p>a) N mineralisation when woolscour sludge was amended with glucose (i) immediately at microcosm set-up and (ii) after woolscour sludge had already been decomposing for 30 days (in a sand matrix with incubation at 22 °C and 20 % moisture for 60 days).</p> <p>b) N mineralisation when woolscour sludge was amended with ammonium nitrate (in a sand matrix with incubation at 22 °C and 20 % moisture for 53 days).</p> <p>c) N mineralisation when woolscour sludge was amended with Fe²⁺ either as ferrous chloride or as part of a trace metal solution (in a sand matrix with incubation at 22 °C and 20 % moisture for 86 days).</p>
<p>H6</p> <p>Decomposition of fellmongery sludge (nitrate leaching)</p>	<p>Surface application of fellmongery sludge to a matrix in a glass leaching columns. Double distilled water was periodically added to the surface, with the drainage water collected and analysed for ammonium and nitrate/nitrite. Incubation was at 22 °C.</p> <p>a) Leach-1: Sand matrix with irregular leaching. Total volume of water added 760 mL and sludge decomposition was for 107 days.</p> <p>b) Leach-2: Sand matrix with regular leaching. Total volume of water added 1460 mL and sludge decomposition was for 109 days.</p> <p>c) Leach-3: Sand matrix with regular leaching and ryegrass seeds scattered over the surface on day 35. The columns were moved into a growth room (22 °C and 16 h photoperiod) once grass germinated. Total volume of water added 1040 mL and sludge decomposition was for 99 days.</p> <p>d) Leach-4: Soil matrix with regular leaching. Total volume of water added 1080 mL and sludge decomposition was for 105 days.</p> <p>e) Leach-5: Soil matrix with regular leaching. Spent columns of Leach-4 were sown with ryegrass, which was harvested after 50 days, with no leachate analysed during this time. The columns were re-amended with fellmongery sludge immediately after ryegrass harvest. Total volume of water added 700 mL and sludge decomposition was for 57 days.</p>
<p>H7</p> <p>Fellmongery sludge as a fertiliser</p>	<p>a) Spent columns of Leach-4 were sown with ryegrass. The columns were moved into a growth room (22 °C and 16 h photoperiod) once grass germinated. The ryegrass was harvested after 50 days and ryegrass dry weight, TN and mineral N determined.</p> <p>b) Ryegrass was harvested from Leach-5 columns on day 20.</p>
<p>H8</p> <p>MB of sludge amended soils</p>	<p>a) Microcosm-scale: Soil was amended with sludges and incubated at 22 °C and 20 % moisture for 50 and 365 days. MB-N was determined using the fumigation-extraction technique.</p> <p>b) Field-scale: Soil was amended in the field with the woolscour and fellmongery sludges. Soil was sampled after 10 months. MB-N was determined on 0-10 cm samples using the fumigation-extraction technique.</p>
<p>H9</p> <p>Response of soil MB to ambient conditions</p>	<p>a) Glasshouse-scale: Plastic pots were filled with soil and placed in the ground, and amended with woolscour and fellmongery sludges at the same time as the remainder of the field was amended. The soil was incubated for 5 months in the field and then 5 months in the glasshouse at 18 °C (± 5 °C). The soil was regularly watered while in the glasshouse. MB-N was determined on 0-10 cm samples using the fumigation-extraction technique.</p>

2.2 THE WOOLSCOUR AND FELLMONGERY SLUDGES

The primary physical difference between the wooldscour sludge and the fellmongery sludge, from their respective industrial sites, were their water contents. The wooldscour sludge was spadable and relatively firm. In the drainage bin at the wooldscour plant (see Figure 1.1), the wooldscour sludge was just firm enough to stand on, sinking about 40 cm into the sludge within 1-2 minutes. At the laboratory, and prior to preparation for long-term storage, wooldscour sludge contained 50 % moisture. The fellmongery sludge was a liquid waste and was run from the bottom of a storage tank (see Figure 1.2) into a collection vessel, however there were definite "clumps" of material within the effluent. In the laboratory, and prior to preparation for long-term storage, fellmongery sludge contained 90 % moisture.

2.2.1 WOOLSCOUR SLUDGE

On the day of wooldscour sludge collection, the sludge had only recently been placed in the drainage bin, which made the sample wetter than normal. Excess moisture was removed by squeezing the sludge between rollers. It was not possible to thoroughly homogenise the wooldscour sludge, due to the grease and fibre constantly forming into matted clumps with the slightest agitation. Several attempts were made to "blend" the sludge; technically this problem was not satisfactorily resolved. The most successful technique was to cut a sub-sample of the squeezed sludge into small fragments, snap-freeze it in liquid N₂, and rub the entire sub-sample through a 3-mm sieve. The <3 mm-sieved sludge was stored frozen (at -14 °C) until required, however agitation of the storage container tended to cause matted clumps to reform. For wooldscour sludge characterisation and decomposition experiments, the sieved material was sub-sampled; any clumps were teased apart, in order to improve sample homogeneity. Therefore the <3 mm wooldscour sludge used for experimental work and analyses was considered the best substrate obtainable at the time, and the care taken at subsampling to use representative material was aimed at further reducing the sample variability. The sludge was kept on ice at all times.

Extra wooldscour sludge was required for amendment of field plots in microbial biomass experiments (Section 2.8.3). This sample was different to the sample collected two years earlier due to modifications of the processing train that had reduced waste

wool fibre, from the dryer, entering the settling ponds. The 1997 sample of woolscour sludge contained less fibre compared to the principal woolscour sludge used for all microcosm work. Results using the second woolscour sludge sample will be referred to as WSc-97 sludge.

2.2.2 FELLMONGERY SLUDGE

The fellmongery sludge was successfully freeze-dried, homogenised and sieved to <2 mm. In the freeze-dried state it was stored in glass containers. Once prepared, this material was easy to handle, although it became very pungent when re-moistened.

2.3 ANALYTICAL METHODS

The sludges were characterised by determining the level of organic matter, grease, C, N, and elemental and heavy metals. Such characterisation would be a normal part of the information required by Councils when determining the rate of sludge application in land-based disposal.

2.3.1 DRY WEIGHT (TOTAL SOLIDS)

The dry weight of samples (in duplicate) was determined by oven drying at 105°C for 24 h. A known weight of sample (1-2 g) was placed in a pre-weighed crucible and oven dried. The crucible was then cooled in a desiccator and re-weighed. Dry weight was calculated according to Equation 2.1.

$$\text{Total Solids (\%)} = (\text{sample dry weight} \div \text{sample wet weight (mg)}) \times 100 \quad \dots(\text{Equ. 2.1})$$

2.3.2 PH

The pH of sludge and soil samples was determined in a 1:4 (v/v; sample: distilled water) slurry, using a Cole Palmer pH meter with a combination glass electrode.

2.3.3 ORGANIC MATTER

Organic matter was determined by weight loss-on-ignition. Oven-dried samples of known weight were ignited in a muffle furnace at 560°C for 6 h. Sample were cooled in a desiccator and re-weighed. Organic matter was calculated as the loss of dry sample weight (Equation 2.2).

$$\text{Organic Matter (\%)} = (1 - (\text{ash weight} \div \text{dry weight (mg)})) \times 100 \quad \dots(\text{Equ. 2.2})$$

2.3.4 GREASE CONTENT

A known weight of sludge was placed in a 20-mL cellulose thimble and placed in the sample chamber of a Soxhlet apparatus, which was then connected to a pre-weighed boiling flask (including anti-bumping granules). Petroleum ether (PE) was added to the sample chamber until one siphon had occurred. Another 50 mL of PE was added to ensure adequate volume of solvent. The sample chamber and flask were connected to the condenser and the whole assembly placed on a thermostatically controlled electric heating mantle. Extraction was performed at 60 °C for 2-3 h. After extraction the solvent was evaporated by standing the flask in a fume cupboard overnight. The grease (in the pre-weighed flask) was then dried for 24 h at 105 °C and the flask re-weighed. Grease content of sludges was calculated according to Equation 2.3. The extraction of woolscour sludge with PE resulted in two distinct fractions of the sludge being produced; a woolgrease fraction (PE soluble material) and a fibre-dirt fraction (PE insoluble material).

$$\text{Grease (\%)} = (\text{grease (mg dry)} \div \text{sludge (mg dry)}) \times 100 \quad \dots(\text{Equ. 2.3})$$

2.3.5 TOTAL CARBON

Total C (TC) was determined using the Walkley-Black method (Hesse, 1971). In a fume cupboard with an asbestos base (or other heat resistant material), 10 mL of K₂CrO₄ (0.17 M) was added to the sample in a 500-mL conical flask, after mixing, 20 mL of concentrated H₂SO₄ was added rapidly. The flask was gently swirled then allowed to stand for 30 minutes. Approximately 200 mL of water plus 10 mL of

phosphoric acid were then added, the flask contents allowed to cool (the reaction is extremely exothermic). Barium diphenylamine sulfonate solution (0.16 %) was added to the flask (5-mL) and titrated against ammonium iron (II) sulphate solution (0.5 M). A blank determination was always done. Calculation of C was according to Equation 2.4.

$$\text{Organic C (\%)} = ((\text{blank titre} - \text{actual titre}) \times 0.3 \times M) \div (\text{weight of oven-dry soil in g})$$

...(Equ. 2.4)

Where M is the concentration of the ammonium iron (II) sulphate solution.

2.3.6 TOTAL NITROGEN

Total nitrogen (TN) N 20 to 30 mg (dw) of sample was determined by semi-micro Kjeldahl digestion, using a salt to acid ratio of 0.6 and a mercury catalyst. Total $\text{NH}_4^+\text{-N}$ was determined by steam distillation after neutralisation of digest with 10 M NaOH- $\text{Na}_2\text{S}_2\text{O}_3$, the distillate was collected in boric acid-indicator solution.

Titration of distillate was against 0.0025 M H_2SO_4 ; 1-mL of acid was equivalent to 70 μg of N, (Equation 2.5 below; Bremner, 1965c). A blank determination was always made, and the titre of this was subtracted from the sample titre. The percent TN for a sample was calculated according to Equations 2.5 and 2.6. Initial TN (i-TN) is the TN added to a microcosm.

$$\text{TN (mg)} = ((\text{sample titre} - \text{blank titre (mL H}_2\text{SO}_4)) \times 0.07 (\text{mg N mL}^{-1} \text{H}_2\text{SO}_4))$$

...(Equ. 2.5)

$$\% \text{ TN} = (\text{TN (mg)} \div \text{sample weight (mg dry)}) \times 100$$

...(Equ. 2.6)

2.3.7 MINERAL NITROGEN

Mineral N species, i.e. ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-), were extracted from a known weight of sample with 2 M KCl (40 mL; Bremner, 1965a). The flask was shaken for 30 minutes at 200 rpm and 30°C. After allowing the sample to settle, 10-mL aliquot of the KCl-extract was placed in a 150-mL flask for steam distillation, with ammonium-N reduced to NH_3 in the presence of MgO, and nitrate-N

and nitrite-N reduced to NH_3 in the presence of MgO and Devarda alloy⁵. Titration was as in section 2.3.6 above.

Direct determination of nitrate-N in plant material was achieved by adding ground, oven-dried (dried at 55 °C for 3 days) plant material directly to 150-mL distillation flasks and adding 10-mL of 2 M KCl (Bremner, 1965a). The suspension was allowed to stand for 30 minutes with periodic swirling to thoroughly wet plant material, with the flask containing the grass being connected to the distillation apparatus. Mineral N was then determined as for mineral N species above.

Ammonia-N evolved during sludge decomposition was analysed by quantitatively rinsing acid trap contents (Section 2.4.7) into distillation flasks, using 10 mL of distilled water. Analysis was by steam distilled after sample neutralisation with 3 mL of 10 M NaOH. Distillate was collected in boric acid-indicator solution and ammonium-N titrated as in section 2.3.6 above.

Together NH_4^+ -N, NO_3^- -N and NO_2^- -N represent the extractable mineral N of a sample or microcosm (Equation 2.7). Mineral-N was usually expressed as a percentage of the initial total N (i-TN) in the substrate (Equation 2.8). Mineralised N included NH_3 -N collected during a specified incubation period and the extractable NH_4^+ -N, NO_3^- -N and NO_2^- -N (Equation 2.9). Net-N mineralisation was the mineral N present after a period of incubation minus the N present in the sample at the beginning of an experiment, and is presented as a percentage of the i-TN (Equation 2.10).

$$\text{Mineral-N (mg)} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+ \text{ (mg)} \quad \dots(\text{Equ. 2.7})$$

$$\text{Mineral-N (\% of i-TN)} = (\text{Mineral-N} \div \text{TN (mg N)}) \times 100 \quad \dots(\text{Equ. 2.8})$$

$$\text{Mineralised-N (mg)} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+ + \text{NH}_3 \text{ (mg)} \quad \dots(\text{Equ. 2.9})$$

$$\text{Net-N Mineralisation (\%)} = (\text{Mineral-N}_{\text{final}} - \text{Mineral-N}_{\text{initial}} \text{ (\% of i-TN)}) \times 100 \quad \dots(\text{Equ. 2.10})$$

⁵ Devarda alloy is a finely divided mixture of zinc, copper and aluminium (50:30:20).

2.3.8 NITROGEN DISTRIBUTION

The distribution of organic N within the woolscour and fellmongery sludges was determined by hydrolysis in 6 M HCl, under reflux, for 24 h at 110 °C. The hydrolysate was filtered, made to volume and analysed using wet chemistry for $\text{NH}_4^+\text{-N}$, hexosamine-N, α -amino acid-N, N that was hydrolysable but not identified, and the non-hydrolysable N (Bremner, 1965c).⁶

2.3.9 INORGANIC ELEMENTS

An oven dried sample was prepared for analysis by ignition in a muffle furnace (560°C for 6 h). The ashed sample was mixed with standard Norrish formula flux and formed into a bead by fusion at 1030 °C. Inorganic elements were determined by standard x-ray fluorescence spectroscopy using a PW1400 spectrophotometer (University of Canterbury Geological Department, 1994).⁷

2.3.10 HEAVY METALS

Samples were prepared for analysis by ignition in a muffle furnace (560°C for 6 h). The ashed sample was digested under reflux for 1 h at 90 °C in a solution containing 4 % nitric acid and 2.5 % perchloric acid. The digest was filtered, made to volume and analysed for Cd, Cu, Ni, Pb, and Zn by flame atomic spectroscopy (Christchurch City Council Waste Management Laboratory, 1994).⁸

2.4 DECOMPOSITION EXPERIMENTS

Three microcosm-scale analyses were used to evaluate the extent to which woolscour and fellmongery sludges decomposed; weight loss (Section 2.4.5), CO_2 evolution (C mineralisation, Section 2.4.6) and N mineralisation (Section 2.4.7). The

⁶ By Dr L.G. Greenfield and Ms Megan Mahan, Department of Plant and Microbial Sciences, University of Canterbury.

⁷ By Mr Steve Brown, Department of Geological Science, University of Canterbury.

⁸ By Mr D. Matthews, Christchurch City Council Bromley Sewage Works, Bromley, Christchurch.

mean (\pm standard error (SE)) is reported for a treatment, on a dry weight basis (dw), as a percentage of the initial weight, C or N, respectively, for the parameter being determined, in triplicate unless otherwise stated.

2.4.1 DECOMPOSITION MICROCOSMS

The physical housing for weight loss microcosms were 30-mL glass centrifuge tubes. Weight loss-microcosms consisted of sample (casein, woolscour sludge or fellmongery sludge) and moisture (including a soil inoculum (Section 2.4.2)). Control weight loss-microcosms were set up as above but chloroform was added (1 mL) to create abiotic conditions. Weight loss-microcosms were covered with plastic film, secured with a rubber band and incubated aerobically at 40 % moisture and 22 °C (\pm 3 °C). Tight fitting rubber bungs were used to cover (seal) the abiotic controls.

The physical housing for C and N mineralisation microcosms were 125-mL glass conical flasks. Carbon and N mineralisation-microcosms consisted of sample, matrix (sand or soil), moisture (including a soil inoculum for sand-based microcosms), amendment as required, and a trap to collect CO₂ (C mineralisation) or NH₃ (N mineralisation). Control mineralisation-microcosms were set up as above, but did not include any sample. Microcosms containing glucose (for C mineralisation) and casein (for weight loss and N mineralisation) were used as examples of substrates of high quality, against which the decomposition of woolscour and fellmongery sludges could be assessed. The glucose and casein were added in a similar manner to the woolscour and fellmongery sludges. The amount of C or N mineralised from the control microcosms was subtracted from the amount of C or N mineralised from the microcosms containing the sample. Microcosms were covered with plastic film, secured with a rubber band and incubated aerobically at 22 °C (\pm 3 °C) and 20 % moisture, unless otherwise stated.

2.4.2 SOIL INOCULUM PREPARATION

Soil inoculum was prepared by addition of 20 g of soil to 60 mL of distilled water. The suspension was stirred and allowed to settle for 5-10 minutes before straining

through fine muslin cloth. Generally 300 μL of filtered soil suspension was added to weight loss and sand-based microcosms.

2.4.3 DETERMINATION OF MOISTURE LEVEL IN MICROCOSM

The moisture level in a microcosm was determined gravimetrically. Based on the dry weight of the sample (Section 2.3.1), the amount of moisture contributed from the sample (and amendment if added) and the inoculum (if added) indicated the additional moisture required for the desired level of moisture. Distilled water was used to bring microcosms up to 40 % moisture for weight loss-microcosms and 20 % moisture for C and N mineralisation-microcosms.

2.4.4 SAND AND SOIL AS DECOMPOSITION MATRICES

Carbon and N mineralisation-microcosms contained 5 g of a matrix, into which the substrate was intimately mixed before the addition of moisture. Two matrices were used; (i) organic matter-free sand, (ii) Ilam silt loam soil (i-TN was 4.6 g N kg^{-1} , with KCl extractable-N at $< 90 \mu\text{g N g}^{-1}$ soil (largely as nitrate)).

- i. Organic matter-free sand was prepared by washing coarse sand several times in distilled water, oven drying and igniting in a muffle furnace for 6 hours at 560°C . This was repeated, with the sand receiving a final wash in distilled water, oven dried and stored until required. Microcosms containing sand are referred to as sand-based microcosms or microcosms with a sand matrix.
- ii. The Ilam soil was taken from the 0-10 cm profile at the University of Canterbury. The soil was air-dried, sieved to $< 2 \text{ mm}$ and stored until required. Microcosms containing the soil are referred to as soil-based microcosms or microcosms with a soil matrix.

2.4.5 ESTIMATION OF DECOMPOSITION BY WEIGHT LOSS

To pre-weighed centrifuge tubes a known weight of sample was added, and the tubes reweighed. Usually, about 1 g of sample was used, on an oven-dried basis and was referred to as the initial weight. Casein was added to microcosms in the same

manner as woolscour and fellmongery sludges were, to indicate weight loss from a high-quality substrate. The contents were moistened with 300- μ L of soil inoculum and distilled water added drop-wise to achieve a final moisture level of 40 % (of the sample dry weight). Control tubes were set up as above, however 1 mL of chloroform was added to prevent microbial activity and the tubes were sealed with rubber bungs rather than covered with plastic film.

The tubes were covered and incubated as required and aerated every five to ten days by removing the cover and blowing a gentle stream of air over the surface, moisture was checked (visually and gravimetrically) and replenished as required. After allotted incubation time, the covers were removed and the tubes were oven-dried at 105 °C for 24 hours. Tubes were cooled in a desiccator and reweighed. Calculation of weight loss is according to Equation 2.11 below.

$$\text{Weight Loss (\%)} = \left(\frac{(\text{Initial sample dry weight (mg)} - \text{Final sample wet weight (mg)})}{\text{Initial sample dry weight (mg)}} \right) \times 100 \quad \dots(\text{Equ. 2.11})$$

2.4.6 ESTIMATION OF C MINERALISATION BY CO₂ EVOLUTION

The diluent used in C mineralisation-microcosms was 5 g of organic matter-free sand. To this was added a known weight of sample, containing approximately 50-mg of total C (TC), and moisture, including 300 μ L of soil inoculum. Glucose was added to microcosms in the same manner as woolscour and fellmongery sludges were, to indicate net-C mineralisation of a high-quality substrate. The amount of C added to the microcosm initially was termed the initial TC (i-TC). An alkaline trap to collect evolved CO₂, containing 2 mL of 1 M NaOH, was suspended in each microcosm. Microcosms were set up in triplicate, covered and incubated at 22 °C and 20 % moisture. Controls microcosms were without sample, but included the matrix and soil inoculum. The CO₂-C evolved from controls was subtracted from the CO₂-C evolved from the microcosms containing the sludges.

After allotted incubation time (82 days), the covers were removed and alkaline traps rinsed (freshly distilled water) into volumetric flasks and made up to volume. Wet-chemistry techniques were used to determine the CO₂ from the traps (Hesse, 1971).

2.4.7 ESTIMATION OF NITROGEN MINERALISATION

The matrices used in N mineralisation-microcosms consisted of 5 g of organic matter-free sand or soil. To this was added a known weight of sample (containing approximately 10 mg of TN), moisture (including 300 μ L of soil inoculum for sand-based microcosms) and amendment where appropriate (Sections 2.6.4, 2.6.5 and 2.6.6). Casein was added to microcosms in the same manner as woollscour and fellmongery sludges were, to indicate net-N mineralisation of a high-quality substrate. The amount of N added to the microcosm initially was termed the initial TN (i-TN). An acid trap to collect evolved NH_3 , containing 1.5 mL of 1 M H_2SO_4 , was suspended in each microcosm. Microcosms were set up in triplicate, covered and incubated at 22 °C and 20 % moisture, unless otherwise stated.

Assessment of woollscour and fellmongery sludge decomposition potential under different environmental conditions involved manipulation of incubation conditions. Mineral N production was determined at different temperatures (5, 10, 22, 25, 43, and 50 °C), different moisture levels (5, 10, 20, and 35 % moisture, which was gravimetrically determined), under anaerobic conditions (Section 2.4.8), and with either sand or soil as the diluent.

After allotted incubation time (50 days for temperature experiment, 53 days for moisture experiment, 108 days for anaerobic experiment and 54 days for different matrices), the covers were removed and acid traps analysed for NH_3 . Ammonia and extractable mineral N were analysed as in Section 2.3.7. Results are reported as net N mineralisation; the mineral N present initially ($\text{Min-N}_{\text{initial}}$) was subtracted from mineral N present after the incubation period ($\text{Min-N}_{\text{final}}$; Equ. 2.10 above).

2.4.8 ESTIMATION OF DECOMPOSITION UNDER ANAEROBIC CONDITIONS

Mineral N production from decomposing woollscour and fellmongery sludges was determined under anaerobic conditions. Anaerobic conditions were produced using AnoCulture kits, which were prepared according to manufacturer's instructions and placed in anaerobic incubation chambers after the microcosms were in place. Microcosms were prepared as in Section 2.4.7, but were left uncovered (see below). The chambers were sealed and incubated at 30 % moisture and 22 °C for 108 days.

Analysis was for mineral N, as in Section 2.3.7, and mineralisation data are presented (Equation 2.10).

Three separate anaerobic chambers were set up; one for control microcosms, one for woolscour sludge microcosms and one for fellmongery sludge microcosms, each chamber containing the treatment in triplicate. Within each chamber, the microcosms were left uncovered during incubation, to ensure that oxygen was effectively removed from the entire atmosphere. Thus, the acid traps absorbed NH_3 from the general headspace of the anaerobic chamber and effectively formed a composite sample (no replication). Had an anaerobic chamber held microcosms of mixed treatments (e.g. fellmongery with control microcosms), the control may have absorbed NH_3 from the fellmongery sludge giving an elevated base level of control mineralisation.

2.5 WOOLSCOUR AND FELLMONGERY SLUDGE INFLUENCE ON NUTRIENT CYCLING

2.5.1 CO-INCUBATION OF WOOLSCOUR AND FELLMONGERY SLUDGES

The effect that decomposing woolscour and fellmongery sludges have on nutrient cycling was estimated by co-incubation of the sludges in sand-based N mineralisation-microcosms (Section 2.4.7), with N contributed in different proportions from the sludges. For example: 20:80-WSc:Fell contained 2 mg woolscour sludge-N and 8 mg fellmongery sludge-N in a microcosm containing a total of 10 mg i-TN. Microcosms were incubated at 20 % moisture and 22 °C for 53 days.

The controls used were woolscour or fellmongery sludge incubated alone. Based on the amount of N mineralised from each sludge independently it was possible to estimate how much N would, in theory, be mineralised by the actual proportion of each sludge in a microcosm. Thus, an experimental amount of mineralised N could be compared to a theoretical amount of mineralised N.

2.5.2 CO-INCUBATION OF FLOWER PETALS WITH WOOLSCOUR OR FELLMONGERY SLUDGE

The effect that decomposing woolsour or fellmongery sludges had on the decomposition of flower petals *Genista* sp. and *Sophora tetraptera* (Kowhai) was estimated by co-incubation in soil-based N mineralisation-microcosms (Section 2.4.7), with 5-mg of N contributed from petals and sludge (total of 10 mg N per microcosm). Microcosms were incubated at 20 % moisture and 22 °C for 30 days.

2.5.3 DECOMPOSITION OF WOOL, WOOLSCOUR SLUDGE OR FELLMONGERY SLUDGES IN SLUDGE-AMENDED SOIL

The effect that woolsour sludge-amended soil (Section 2.8.3) had on N mineralisation during the decomposition of different sources of wool was determined using the amended soil for soil-based N mineralisation-microcosms (Section 2.4.7). The wool samples were raw wool (cut from over the kidney region of a four year-old ram), scoured wool (scoured using the mini-bowl system and provided by WRONZ) and woolsour sludge.

The effect that fellmongery sludge-amended soil (Section 2.8.3) had on N mineralisation during the decomposition of fellmongery sludge was determined using the amended soil for soil-based N mineralisation-microcosms (Section 2.4.7). Microcosms were incubated at 20 % moisture and 22 °C for 30 days.

2.6 CONSTRAINTS ON WOOLSCOUR SLUDGE DECOMPOSITION USING N MINERALISATION

2.6.1 THE EFFECT OF SOIL INOCULUM FROM SOIL TREATED LONG-TERM WITH WOOLSCOUR WASTES

A soil inoculum was prepared as in Section 2.4.2 using a soil taken from the 0-10 cm profile of a sheep- and cattle-grazed paddock that had been receiving woolsour wastes from an Ashburton woolsour operation for >12 years (treated inoculum). The control was an inoculum prepared as in Section 2.4.2. Sand-based N mineralisation microcosms were used to determine the effect that the treated soil inoculum had on

decomposition of woollscour sludge. Incubation was at 22 °C and 20 % moisture, with analyses at 50, 90 and 162 days.

2.6.2 THE EFFECT OF WOOLGREASE ON PROTEIN DECOMPOSITION

Woolgrease, extracted from woollscour effluent using hexane, was provided courtesy of WRONZ. The woolgrease and casein were mixed until homogeneous by grinding using a mortar and pestle. Thus the final admix was an homogeneous substrate of casein and woolgrease, which was then added to N mineralisation microcosms as in Section 2.4.7, and incubated at 22 °C and 20 % moisture, with analysis for mineralised N was made after 116 days.

2.6.3 DECOMPOSITION OF THE FIBRE-DIRT FRACTION OF WOOLSCOUR SLUDGE

The fibre-dirt fraction of woollscour sludge (Section 2.3.4) was decomposed in sand-based N mineralisation microcosms (Section 2.4.7). Incubation was at 22°C and 20 % moisture, with analyses at 10, 34, 50, 90 and 162 days.

2.6.4 THE EFFECT OF GLUCOSE AMENDMENT ON WOOLSCOUR SLUDGE DECOMPOSITION

Nitrogen mineralisation-microcosms (Section 2.4.7) were used to estimate the decomposition of woollscour sludge amended with glucose in two separate experiments. Firstly, glucose was added at the setting-up stage of the decomposition experiment; amendment was at 1 and 10 % (w/w) of woollscour sludge (dry weight basis). Incubation was for 50 days in sand-based microcosms. Secondly, in soil-based microcosms glucose was added after woollscour sludge had been decomposing for 30 days and then incubation continued for a further 30 days; amendment was at 10 and 25 % (w/w) of woollscour sludge (dry weight basis). Glucose was added in solution. Incubation was at 20 % moisture and 22 °C.

2.6.5 THE EFFECT OF AMMONIUM NITRATE AMENDMENT ON WOOLSCOUR SLUDGE DECOMPOSITION

Nitrogen mineralisation-microcosms (Section 2.4.7) were used to estimate the decomposition of wooldscour sludge amended with ammonium nitrate, with N contributed in different proportions from the wooldscour sludge or ammonium nitrate (AN). For example: 20:80-WSc:AN contained 2 mg wooldscour-N and 8 mg of N from NH_4NO_3 in a microcosm containing a total of 10 mg i-TN. Microcosms were incubated at 20 % moisture and 22 °C for 34 and 53 days.

The control was wooldscour sludge incubated alone. From the amount of N mineralised from wooldscour sludge it was possible to estimate how much N, in theory, would be mineralised by the amount of sludge in an ammonium nitrate-amended microcosm. Mineralisation was determined (Equation 2.10), allowing experimental and theoretical amounts of mineralised N to be compared.

2.6.6 THE EFFECT OF TRACE METALS AMENDMENT ON WOOLSCOUR SLUDGE DECOMPOSITION

Nitrogen mineralisation-microcosms (Section 2.4.7) were used to estimate the decomposition of wooldscour sludge amended with iron (II) added either alone or as part of a solution of trace metals (Table 2.7). Amendment was at 0, 8.7, 87.0, 870.0, 8700.0 ppm of iron (II) to wooldscour sludge (on a dry weight basis). Microcosms were incubated at 20 % moisture and 22 °C for 86 days.

Table 2.7. Trace metal solution used to amend wooldscour sludge.

Trace Metal	Concentration µg per µL
$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	18.00
H_3BO_3	5.00
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	2.50
ZnCl_2	1.00
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.70
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	0.30
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.15
$\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$	0.10
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.10

2.7 CONSEQUENCES OF FELLMONGERY SLUDGE DECOMPOSITION - LEACHED MINERAL N

2.7.1 LEACHING SET UP

The physical housing for fellmongery sludge leaching-microcosms were glass columns 5.1-cm internal diameter and 15-cm depth. The tapered region, 3-cm depth, was filled with organic matter-free sand and separated from the matrix by GF-A filter paper. The drainage tube was plugged using GF-A filter paper to prevent sand from dislodging. The matrix (dry) was poured into the columns, with gentle tapping to distribute evenly, filling the straight portion of the column to a depth of 9-cm (packing material was 184 cm³ in volume and 20.43 cm² surface area, which was equivalent to 2.04 x 10⁻⁷ ha). This left an area of 3-cm for addition of distilled water used to leach the columns (Figure 2.13).

The matrix was coarse, washed river sand (organic matter of 0.5 %) or sandy soil (Ilam soil mixed 50/50 with river sand). Each experiment consisted of six packed columns arranged on a rack, which allowed 100-mL beakers to be placed underneath each column to collect leachate. Prepared (unamended) columns were leached three times with 100-mL of distilled water over a period of five days, columns were allowed to stand for another five days prior to amendment with fellmongery sludge.

The columns were set up, three for amendment with fellmongery sludge and three as non-amended controls, and randomly located relative to each other. Fellmongery sludge (735 mg), containing 71 mg of TN (equivalent to 350 kg N ha⁻¹), was spread evenly over the soil surface and 20 mL of distilled water added to remoisten the sludge. This was insufficient water to cause displacement of water already on the column.

Leaching was with double distilled water, added in three or four portions, with all columns receiving equal water per leaching event (added volume per leaching ranged from 40 to 100 mL). The columns were allowed to drain overnight. The leachate was analysed for water-soluble mineral N, with ammonium-N and nitrate/nitrite-N determined separately (Section 2.3.7). Incubation was at 22 °C, and when ryegrass was included as a treatment the columns were placed in a growth room at 22 °C with a 16-h photoperiod.



Figure 2.13. A leaching-microcosm; packed with sand and amended with fellmongery sludge, (at a rate equivalent to 350 kg N ha^{-1}).

The amount mineral N leached from the unamended control columns was subtracted from the mineral N leached from the amended columns. This gave net-mineral N leached from fellmongery sludge. Results are reported on a dry weight basis as the percent of applied N (i-TN) for each individual leaching and for the cumulative amount of mineral N leached.

2.7.2 LEACHING EXPERIMENTS

Five leaching experiments were set-up, Leach-1, -2, -3, -4 and -5. Leach-1, Leach-2 and Leach-3 had sand as the packing matrix and Leach-4 and Leach-5 had sandy soil as the packing matrix.

Leach-1 was aimed at determining how much water-soluble mineral N was leached during the decomposition of fellmongery sludge, and whether nitrate was the principal mineral N species i.e. addressing hypothesis H6 (Table 2.5). There were 10 individual leachings, with a cumulative volume of 760 mL of distilled water added (Table 2.8).

Leach-2 was aimed at determining whether the amount of mineral N, specifically nitrate, leached during the decomposition of fellmongery sludge was influenced by how much water was added to the columns. There were 21 individual leaching, with a cumulative volume of 1460 mL of distilled water added (Table 2.8).

Leach-3 was aimed at determining whether the amount of mineral N, specifically nitrate, leached during the decomposition of fellmongery sludge was reduced when grass was present. The fellmongery sludge was applied to the sand and allowed to decompose for 35 days. After the columns had drained on day 35, ryegrass seeds were sprinkled over the surface (40 seeds per column) and the experiment continued with regular leachings. There were 14 individual leachings, with a cumulative volume of 1040 mL of distilled water added (Table 2.8). The ryegrass was harvested at the termination of Leach-3 (above ground material only), by cutting the grass blades approximately 20 mm above the sand surface. The ten longest blades of grass were used from each column to determine the above ground plant material produced (dry weight basis).

Leach-4 was aimed at determining whether soil, rather than sand, as the matrix used to pack the columns affected the amount of mineral N, specifically nitrate, leached during the decomposition of fellmongery sludge. Ilam soil was mixed 50/50 with the washed river sand and this mixture was used to pack the leaching columns. The columns were amended with fellmongery sludge and regularly leached. There were 12 individual leaching, with a cumulative volume of 1080 mL of distilled water added (Table 2.8).

Leach-4 was terminated after 105 days. However, unlike the other three leaching experiments, the columns were not unpacked and washed. Rather, 40 ryegrass seeds were scattered over the surface of each column. The columns were regularly watered but no leachate was collected during the growth of the ryegrass. The aim of this experiment, intermediate between Leach-4 and Leach-5, was to determine whether soil previously amended with fellmongery sludge, and heavily leached, could produce more ryegrass than the heavily leached non-amended control soils. The grass (above ground material only – the roots were left in the soils to permit the grass to grow for Leach-5) was harvested after 50 days by cutting 20-mm above the soil surface. Total N, nitrate-N and dry weight were determined on the total grass harvested for each column. The columns were retained for Leach-5.

Leach-5 was aimed at determining whether soil re-amended with fellmongery sludge and with ryegrass present, reduced the amount of nitrate leached. Leach-5 ran for only 57 days, during which time there were 7 individual leaching, with a cumulative volume of 800 mL of distilled water added (Table 2.8). The ryegrass was harvested as above after 20 days and allowed to re-grow. The experiment was terminated early because all the grass in the amended columns (but not the unamended control columns) all died after the harvest on day 20, hence the control and treatment columns were no longer valid comparisons.

Table 2.8. Average cumulative volume of water added and collected from the leaching columns. Leach-1, -2 and -3 were sand-packed columns; Leach-4 and Leach-5 were soil-packed.

	Leaching Events (#)	Cumulative Volume Added (mL)	Cumulative Volume Collected (mL)
Leach-1 (sand, irregular leaching)	10	760	620
Leach-2 (sand, regular leaching)	21	1460	1317
Leach-3 (sand, regular leaching, ryegrass)	14	1040	744
Leach-4 (soil, regular leaching)	12	1080	916
Leach-5 (soil, re-amendment, regular leachings, ryegrass)	7	800	367

2.8 MICROBIAL BIOMASS OF WOOLSCOUR AND FELLMONGERY SLUDGE-AMENDED SOILS

2.8.1 MICROBIAL BIOMASS METHODOLOGY

Microbial biomass N (MB-N) was estimated by the fumigation-extraction method (Brookes *et al.*, 1985a; Brookes *et al.*, 1985b), except ethanol was not removed from the chloroform. Briefly, ~60 g of moist soil (~25 % moisture by weight) was divided into two portions of known weight, one for fumigation and one for immediate extraction. Extraction was with 80 mL of 0.5 M K₂SO₄ in a 250-mL conical flask, soil and K₂SO₄ were shaken for 30 minutes at 30 °C and 200 rpm, before filtering through GF-A filter paper under vacuum. Fumigation was for 24 hours with CHCl₃, after which time the fumigated soil was extracted as above with K₂SO₄. Three other portions of the soil sample were taken to determine TN, dry weight (and organic matter), and pH.

The TN of the filtered K₂SO₄ extract (10-mL aliquot) was determined using the Kjeldahl method. The aliquot was acidified with 1.5 mL H₂SO₄ and heated to evaporate the water, which took about one hour. Mercuric oxide (0.4 g) was added to cool, evaporated samples. Digestion for TN was for 1½ hours, or until clear; titration for TN as in Section 2.3.6.

Calculations for MB-N are in Equations 2.12, 2.13, and 2.14 below. Flush-N is the amount of TN present due to fumigation (Equation 2.12), and MB-N is the flush-N corrected for the actual efficiency of the fumigation (k_{EN}) process, the absolute MB ($\mu\text{g MB-N g}^{-1}$ soil; Equation 2.13). Microbial biomass results are also reported as MB-N (% of soil N; Equation 2.14). The latter is the form preferred by the author since it indicates the efficiency of microbial immobilisation of soil N and is appropriate when the materials added have the potential to significantly increase soil N. MB-N (% of soil N) is similar to the microbial quotient used, except for N as the parameter determined not C (Sparling, 1997).

$$\text{Flush-N } (\mu\text{g N g}^{-1} \text{ soil}) = ((\text{TN in extract from fumigated soil}) - (\text{TN in extract from non-fumigated soil})) \dots (\text{Equ. 2.12})$$

$$\text{MB-N } (\mu\text{g MB-N g}^{-1} \text{ soil}) = (\text{flush-N} \times k_{EN}) \dots (\text{Equ. 2.13})$$

$$\text{where } k_{EN} = 0.35 \text{ (Ross, 1992)}$$

$$\text{MB-N (\% of soil N)} = (\text{MB-N} \div \text{initial-TN}) \times 100 \quad \dots (\text{Equ. 2.14})$$

2.8.2 MICROCOSM STUDY

The aim of the microcosm-scale MB experiment was to determine whether amendment of soil with woolscour and fellmongery sludges altered the MB (H8 in Table 2.5), which was estimated using the fumigation-extraction method. Microbial biomass microcosms were plastic pottles, 11-cm high x 5-cm wide (surface area equivalent to 1.3×10^{-7} ha), into which 60 g of air-dried soil were placed. The soil was amended with fellmongery (270 mg dw) or woolscour sludge (580 mg dw) equivalent to 200 kg N ha^{-1} , and incubated at 22°C and 20 % moisture for 50 and 365 days before analysis for MB-N (Section 2.8.1). The soil was regularly mixed, aerated, moistened and any seeds that germinated carefully removed.

2.8.3 FIELD PLOT STUDY

The aim of the field-scale MB experiment was to determine whether amendment of soil with woolscour and fellmongery sludges altered the MB, which was estimated using the fumigation-extraction method. An area of 64 m^2 was rotary hoed and the central area divided into three strips (one for woolscour sludge, one for fellmongery sludge and one for control treatment). Each strip contained nine 50×50 -cm plots, with a buffer zone of 50 cm in width between strips. Within a treatment strip, alternative 50×50 -cm plots of soil were removed to a depth of 20 cm and the soil equally packed into $4 \times (17\text{-cm}^2 \times 18\text{-cm deep})$ plastic containers. The four plastic containers were relocated in the hole and the surplus soil packed around each container to fill the hole. When the containers were correctly placed, the soil surface showed only a narrow lip of the each plastic container in the alternative 50×50 plots. Four sets of four containers were used per treatment strip (Figure 2.14). The plot was allowed to settle for six months, with periodic hand weeding.

Attempts to keep the area weed free were abandoned, with weeds considered the “crop”, with periodic weeding equivalent to “harvesting plant material”. The area was cleared of weeds before removal of the potted soil after five months (Section 2.8.4) and again before sampling for MB estimation after 10 months. Soils were sampled 0-10 cm depth and stored at 5 °C until analysis (within three days). Microbial biomass was determined according to Section 2.8.1.

2.8.4 GLASSHOUSE STUDY

The aim of the glasshouse study was to determine whether the MB of soil amended with wooldscour and fellmongery sludges (i) responded to the mesophilic incubation conditions of the glasshouse by increasing in quantity and (ii) whether the response of the MB was similar to the non-amended control soil. The soils used in the glasshouse trial were the potted field soils, which were removed from the field plot after five months at field conditions (Section 2.8.3) and placed in the glasshouse for a further five months of incubation (18 °C \pm 5 °C and regular watering). Soil was sampled as in Section 2.8.3 and analysed for MB-N as in Section 2.8.1.

The weed crop that re-grew during incubation in the glasshouse was harvested and dry weight (plant material was dried at 55 °C for seven days) and TN determined (Section 2.3.6). No attempt was made to identify the plants present.

2.9 STATISTICAL ANALYSIS

2.9.1 DECOMPOSITION EXPERIMENTS

The level of replication ($n = 2$ or 3) during decomposition experiments was generally insufficient to allow meaningful statistical analysis.

2.9.2 MICROBIAL BIOMASS EXPERIMENTS

Analysis of variance (ANOVA; Microsoft Excel, 1997) was used to compare the means of soil amended with wooldscour sludge, fellmongery sludge and the unamended control ($n = 5$ or 6). When a significant difference between the three means was found

($\alpha < 0.05$), the means were compared using Duncan's multiple-range test (Walpole, 1974). Differences between means were signified by; NS = not significant, $p = < 0.05$, $p = < 0.01$ and $p = < 0.001$.

3 RESULTS

3.1 PHYSICAL AND CHEMICAL PROPERTIES OF WOOLSCOUR AND FELLMONGERY SLUDGES

The woolscour sludge sample, sieved to < 3 mm, had 35 % moisture. However, the moisture level tended to fall with long-term frozen storage and later samples had ~20 % moisture (Table 3.9). Within the frozen sample there was a high degree of heterogeneity and considerable effort was made at each subsampling to thoroughly mix the woolscour sludge. The heterogeneity of woolscour sludge was seen in results obtained during decomposition experiments, which tended to show more variation between replicates than was seen for fellmongery sludge. Fellmongery sludge was readily freeze-dried and the powder ground and sieved to be < 2 mm. Freeze-dried fellmongery sludge had steady moisture content throughout the experimental work (Table 3.9). Fellmongery sludge had elevated levels of Na and S in its inorganic fraction, both of which are present in chemicals added during the fellmongering process (Table 3.10).

The woolscour sludge contained 62 % organic matter. After Soxhlet extraction of the sludge with petroleum ether two fractions were recovered; the woolscour grease fraction (woolgrease), which contained 0.1 % TN and 99 % organic matter, and the fibre-dirt fraction. The fibre-dirt fraction had 4.7 % TN and accounted for essentially all the inorganic component of woolscour sludge (Table 3.10). The organic fraction of woolscour sludge contained 53 % woolgrease and 47 % fibre-dirt (ash-free; Table 3.11).

There was a discrepancy between the TN calculated on an ash-free basis for woolscour sludge as a whole and when calculated from the grease and fibre-dirt fractions individually. Together the two fractions contained 2 % less TN than the untreated material (Table 3.11). This suggested that the treatment with petroleum ether had removed some N-containing constituents of the wool fibre (denatured the fibre-dirt fraction; Section 4.3.2).

The observable difference between woolscour sludge (used for essentially all the experimental work) and the WSc-97 sludge (used to amend the field plots; Section 2.8.3) was that WSc-97 sludge had less fibre and was more like greasy mud. These observations are reflected in the TN, total solids, and organic matter of WSc-97 (Table 3.9).

Table 3.9. Physical and chemical characteristics of woolscour and fellmongery sludges as used in the experimental work.

Characteristic	Units	Woolscour Sludge	WSc-97 ¹	Fellmongery Sludge
pH		7.0	7.0	5.2
Total Solids	%	65-80 ¹	43.1	7
Organic Matter	% dry wt	62	38	79
Grease	% dry wt	33	—	6
Total Carbon	% dry wt	37.3	—	44.4
Total Nitrogen	% dry wt	4.5	0.87	9.7
C:N ratio		8.3	—	4.6
Mineral N	% total N	4.3	—	2.9
Hexosamine-N	% total N	< 1	—	0
Hydrolysable Unidentified N	% total N	28	—	30
α -Amino Acid-N	% total N	55	—	58

¹ Moisture content was 35 % initially but gradually declined with sample storage to 20 % by the end of experimental work.

Table 3.10. Elements present in woolscour and fellmongery sludges.

Element	Woolscour Sludge	Woolscour Fibre & Dirt	Fellmongery Sludge	Soil ^a
	% of Ash			
SiO ₂	71.58	73.32	48.67	75.00
TiO ₂	0.52	0.45	0.45	0.72
Al ₂ O ₃	11.66	11.48	8.27	9.68
Fe ₂ O ₃	2.96	2.74	6.39	3.44
MnO	0.05	0.04	0.21	0.11
MgO	0.93	0.87	0.95	0.85
CaO	4.74	3.92	5.83	1.33
Na ₂ O	3.42	3.55	11.09	0.96
K ₂ O	1.90	1.78	3.11	1.78
P ₂ O ₅	0.24	0.22	2.77	0.11
SO ₃	1.60	1.12	11.68	0.10

^a Data courtesy of Dr L.G. Greenfield, Department of Plant and Microbial Science, University of Canterbury.

Table 3.11. Woolscour sludge (WSc) after extraction with petroleum ether, showing the two fractions produced (Grease and Fibre/Dirt (Fibre)) and the TN contributed from each, on an ash-free basis (AF).

100 mg WSc _(AF)	⇒	53 mg Grease _(AF)	+	47 mg Fibre _(AF)	
↓		↓		↓	
@ 7.21 % TN _(AF)		@ 0.1 % TN _(AF)		@ 10.80 % TN _(AF)	
↓		↓		↓	
7.21 mg TN		0.05 mg TN	+	5.08 mg TN	= 5.13 mg TN

The level of metals in fellmongery sludge was less than that recommended by the Christchurch City Council for material applied to agricultural land (Table 3.12). While most of the metals associated with woolscour sludge were below the recommended level, Zn was at the maximum level and Cd was more than twice the recommended maximum (Table 3.12).

Table 3.12. Heavy metals present in woolscour and fellmongery sludge on a dry weight basis.

Metal	Woolscour Sludge	Fellmongery Sludge	Maxima for Agricultural Land Application ^a
	(ppm)		
Copper	8	55	140
Chromium	7	13	600
Nickel	8	11	35 ^b
Zinc	308	129	300
Cadmium	7	2	3
Lead	10	12	300

^a Recommended maximum for composts applied to agricultural land (Personal communication from Dr D. Matthews, Water Treatment Works, Christchurch City Council).

^b 75 ppm (Fliebbach *et al.*, 1994)

3.2 EXPERIMENTAL SCHEME AND RESULTS

The relationship between the hypotheses stated and tested during this research (Table 2.5 and Table 2.6) and the experimental results is shown in Figure 3.15.

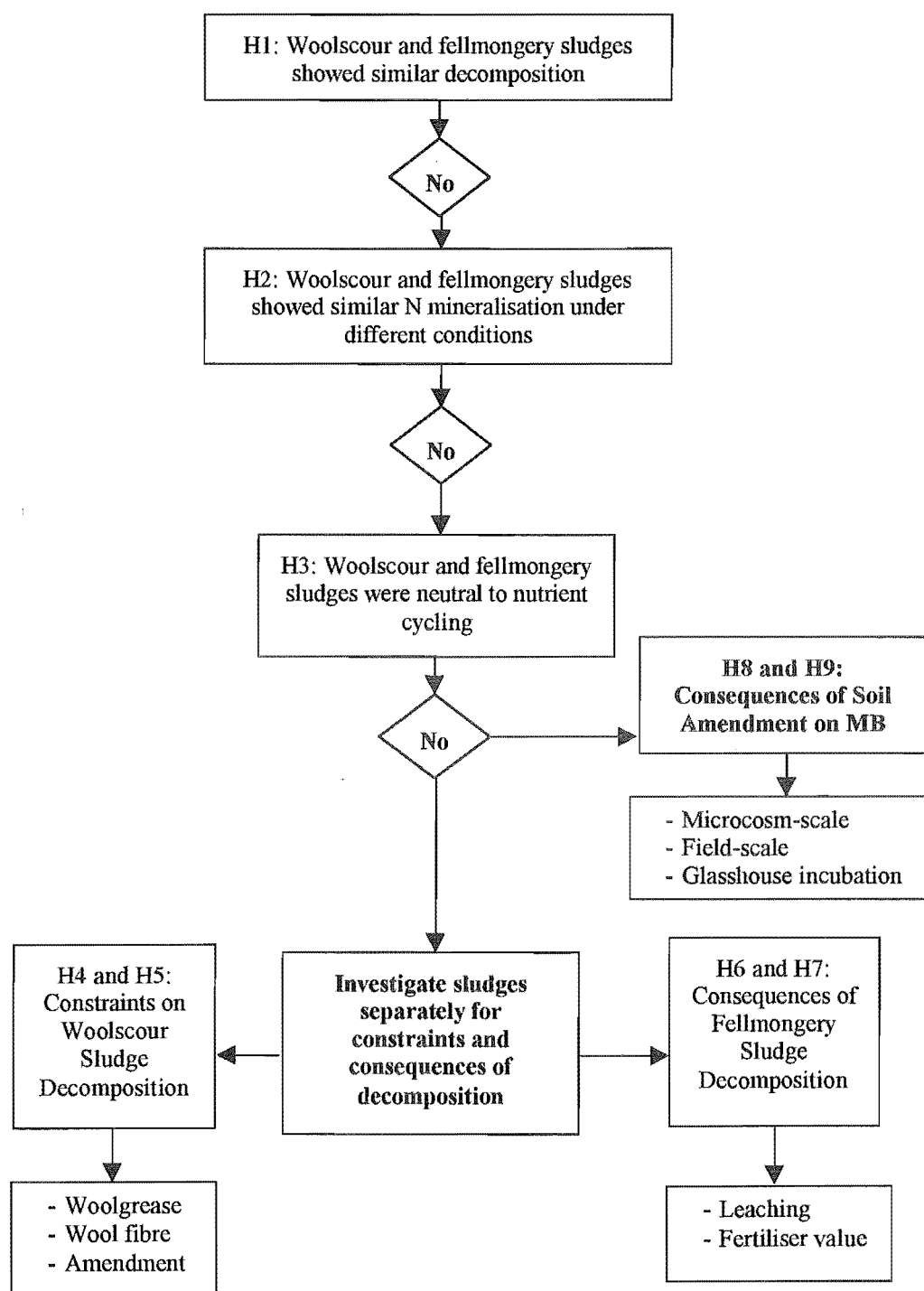


Figure 3.15. Flowchart of the experimental sequence and results from the study into the decomposition of woolscur and fellmongery sludges.

3.3 DECOMPOSITION OF WOOLSCOUR AND FELLMONGERY SLUDGES

The dictionary definition of a microcosms is, (1) a miniature representation or (2) a community or complex unity viewed in this way⁹. Microcosm-scale experiments were used to study the decomposition of woolscour and fellmongery sludges. Although microcosm studies are recognised as being artificial and results cannot be directly extrapolated to field conditions, they permit decomposition potential to be determined. The decomposition of woolscour and fellmongery sludges was determined using weight loss, CO₂ evolution and N mineralisation. The effect of different incubation temperatures and moisture, and anaerobic atmosphere on woolscour and fellmongery sludge decomposition was determined using N mineralisation. Changing the matrix for N-mineralisation microcosms from organic matter-free sand to soil (Section 2.4.4), assessed the influence of soil on potential N mineralisation of the sludges.

3.3.1 ESTIMATION OF DECOMPOSITION BY WEIGHT LOSS

The aim of this experiment was to determine whether woolscour and fellmongery sludges would decompose under control conditions (22 °C and 40 % moisture). After 40 days of decomposition, casein had lost 56 ± 2 % of its initial weight, which was considerably more than that lost by woolscour (3 ± 3 % of its initial weight) or fellmongery sludge (38 ± 1 % of its initial weight). After 50 days decomposition, woolscour sludge had lost 10 ± 1 % and fellmongery sludge 38 ± 0 % of their initial weight (Figure 3.16).

The abiotic controls for casein and fellmongery sludge showed essentially no weight change from their initial weight after 40 days decomposition. However, the abiotic controls of woolscour sludge showed similar variation for weight loss (and gain) as the non-chloroformed samples (Table 3.13). The abiotic controls that showed a loss of weight suggested that these microcosms were not abiotic, even though they had a distinctive smell of chloroform. The apparent weight gain during woolscour sludge decomposition was postulated to be due to the grease fraction of woolscour sludge

⁹ (The Oxford English Dictionary, 1996)

retaining or trapping moisture in some way during drying at 105 °C, i.e. an artefact of the weight loss method using such a greasy sample.

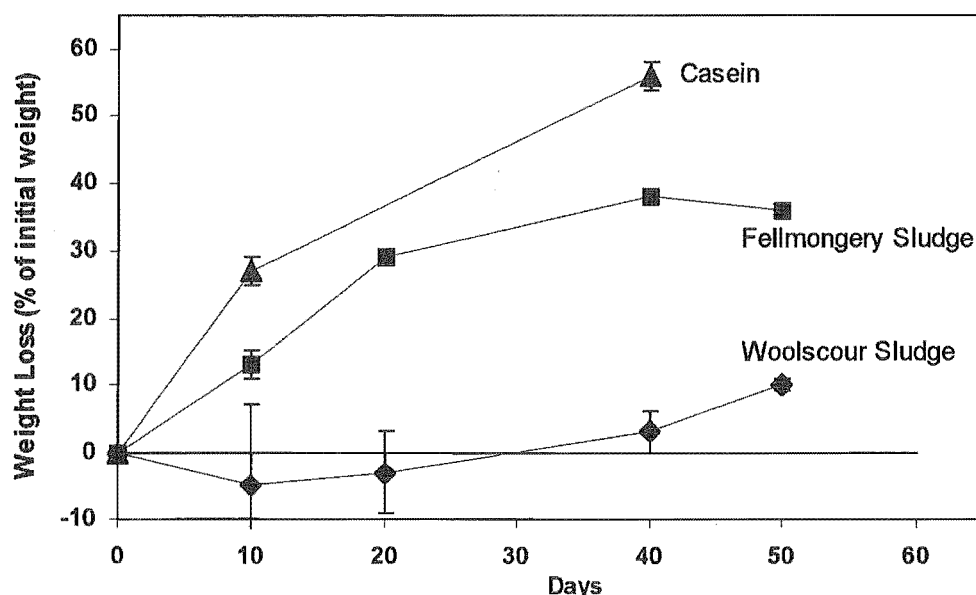


Figure 3.16. Weight loss during the decomposition of casein (▲), woolscour (◆) and fellmongery (■) sludges under biotic conditions (% of initial dw). Incubation was at 40 % moisture and 22 °C (± 3 °C). Means (\pm SE), $n=3$, are presented.

The postulate was confirmed by an experiment that involved the addition of 5 g of water to 5 g of woolgrease, drying at 105 °C for 24 h and cooling in a desiccator. The weight loss averaged of 2.5 ± 0.5 g ($n = 6$), which indicated that not all the added water had been removed. On cooling, the woolgrease formed a continuous layer over the remaining water, which was still visible even after further drying treatments at 105 °C. Control tubes containing only woolscour grease lost 0.04 % of their initial weight. Hence, the grease fraction of woolscour sludge had the potential to retain added moisture, suggesting that weight loss was not a satisfactory method to quantify decomposition of woolscour sludge and the results presented in Figure 3.16 must be considered an inaccurate description of woolscour sludge decomposition. The large weight fluctuations seen during the decomposition of woolscour sludge were considered an artefact of the weight loss method.

Table 3.13. Individual replicates of wooldscour sludge during weight loss experiment. The net decrease (Δ^-) and increase (Δ^+) in weight are shown. Incubation was at 20 % moisture and 22 °C (± 3 °C).

Days	Biotic			Abiotic		
	% of initial weight (net change)					
10	18.5 (Δ^-)	18.8 (Δ^+)	14.9 (Δ^+)	1.2 (Δ^-)	18.2 (Δ^-)	12.6 (Δ^+)
20	11.1 (Δ^+)	9.2 (Δ^-)	5.7 (Δ^+)	ND ^a	ND	ND
40	6.4 (Δ^-)	0.4 (Δ^-)	broken	11.7 (Δ^-)	3.2 (Δ^+)	ND
50	10.7 (Δ^-)	10.1 (Δ^-)	8.2 (Δ^-)	ND	ND	ND

^a ND = not determined.

3.3.2 ESTIMATION OF DECOMPOSITION BY CO₂ EVOLUTION

The aim of this experiment was to determine whether wooldscour and fellmongery sludges would mineralise C under constant conditions (22 °C and 20 % moisture; Figure 3.17). The evolution of CO₂ during decomposition is a direct measure of the C mineralised by microorganisms and is presented here as a percentage of the initial substrate total C (i-TC). After 40 days of decomposition, glucose had mineralised 52 ± 3 % i-TC, considerably more than mineralised from decomposing fellmongery sludge (37 ± 1 % i-TC) or wooldscour sludge (18 ± 1 % i-TC). After 82 days of decomposition, fellmongery sludge had mineralised 42 ± 2 % i-TC and wooldscour sludge 30 ± 1 % i-TC (Figure 3.17).

3.3.3 ESTIMATION OF DECOMPOSITION BY NITROGEN MINERALISATION

The mineralisation of N represents extensive decomposition of organic material and is a reliable method for assessing N released from cellular material, especially proteins. The mineral N released may be immobilised by plants, microbes or adsorbed on inorganic material, or remain mobile and eventually be leached from the matrix. The aim of this experiment was to determine whether wooldscour and fellmongery sludges would mineralise N under ambient conditions (22 °C and 20 % moisture). Mineralisation data are presented (Equation 2.10) such that any initial mineral N in the

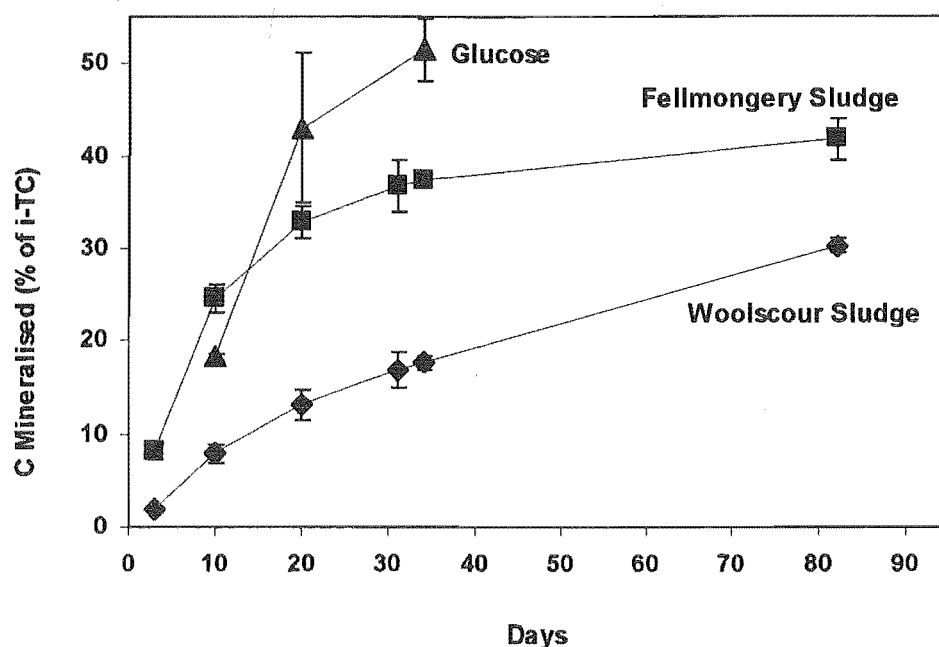


Figure 3.17. Carbon mineralisation during decomposition of glucose (▲) and wooldscour (◆) and fellmongery (■) sludges. Incubation was at 20 % moisture and 22 °C (± 3 °C) for 82 days in sand-based microcosms. Means (\pm SE), $n=3$, ($n=2$ for glucose) are presented.

wooldscour or fellmongery sample has been subtracted from the mineral N present after a designated incubation period.

Over a range of N mineralisation experiments conducted over four years, wooldscour sludge had initial-mineral N ranging from 2 to 7 % of i-TN, with an average of 4 ± 0 % i-TN ($n = 32$; Figure 3.18 (a)). Fellmongery sludge had initial-mineral N ranging from 1 to 3 % of i-TN, with an average of 2 ± 0 % of i-TN ($n = 18$; Figure 3.18 (b)). The slope of the trend lines on Figure 3.18 are essentially zero, which indicated that sludges did not alter in their initial mineral N content over the storage period for the research.

The control incubation conditions for decomposition experiments were an aerobic atmosphere, 20 % moisture and 22 °C. Within any one experiment, a treatment level (e.g. variation in temperature or moisture) was compared to the amount of N mineralised from the control microcosms of that specific experiment. Therefore, a broad picture of the net-N mineralised during the decomposition of wooldscour and fellmongery sludges, in a sand matrix, was gained by plotting the control data in a

composite graph (Figure 3.19). The number of individual determinations at any specific incubation time ranged from 2 to 10. It was particularly important for the net-N mineralised during the decomposition of wooldscour sludge to be carefully assessed, since considerable variation occurred during its decomposition under the control conditions (see Section 3.4 below).

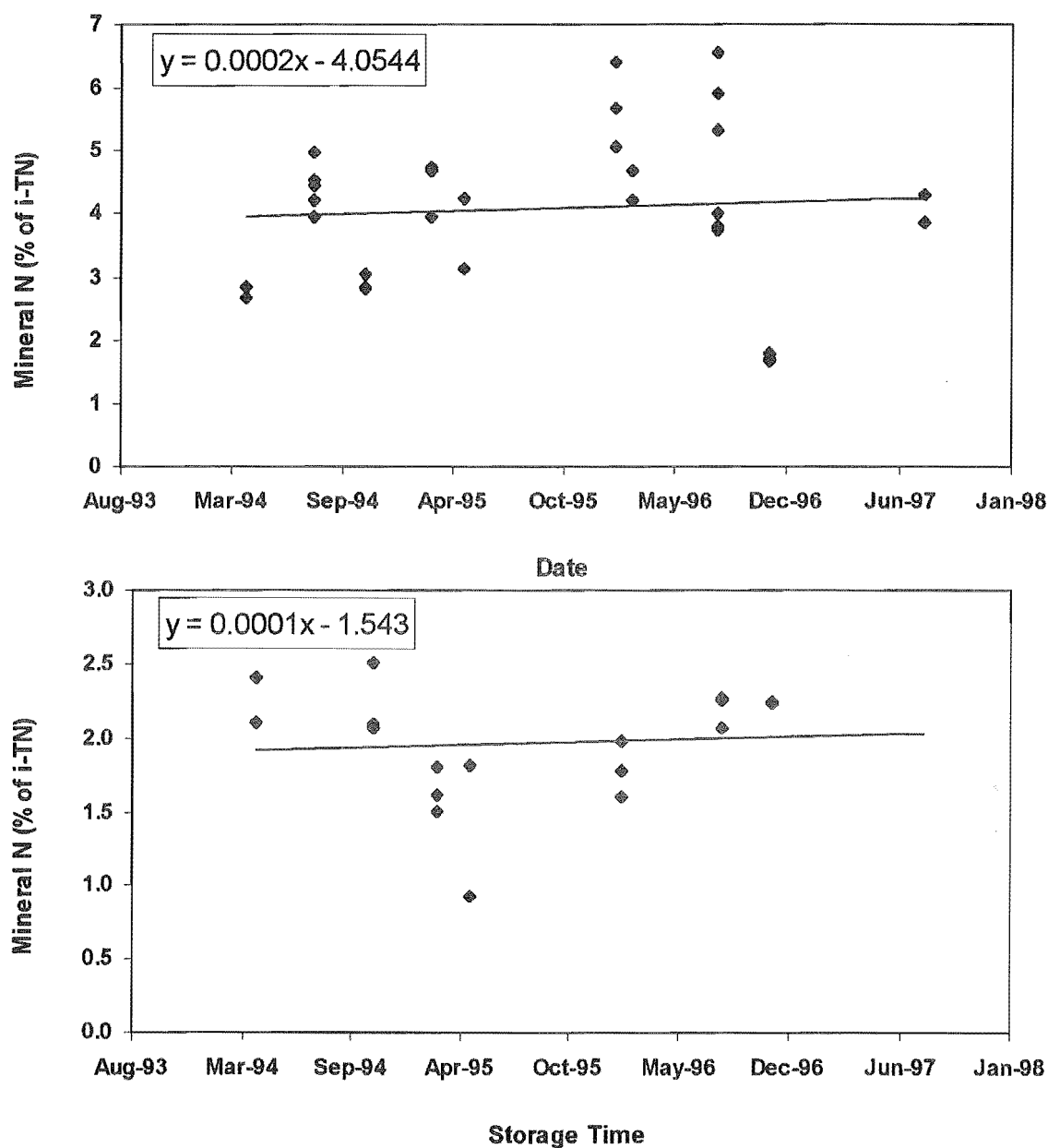


Figure 3.18. KCl-extractable mineral N present on Day = 0 of (a) wooldscour sludge and (b) fellmongery sludge N mineralisation experiments.

After 34 days of decomposition, net-N mineralisation from woolscour sludge ranged from 5 to 15 % of i-TN, with an average of 9 ± 1 % of i-TN ($n = 9$; Figure 3.19). The fellmongery sludge had a range of N mineralisation from 44 to 55 % of i-TN, with an average of 51 ± 1 % of i-TN ($n = 9$; Figure 3.19). After 50 days of decomposition, net-N mineralisation from woolscour sludge ranged from 2 to 25 % of i-TN, with an average of 9 ± 2 % of i-TN ($n = 9$). After 50 days of decomposition, the fellmongery sludge showed a range of N mineralisation from 37 to 60 % of i-TN, with an average of 53 ± 2 % of i-TN ($n = 10$; Figure 3.19). The longer incubation period for woolscour sludge did not result in additional N being mineralised (Figure 3.19).

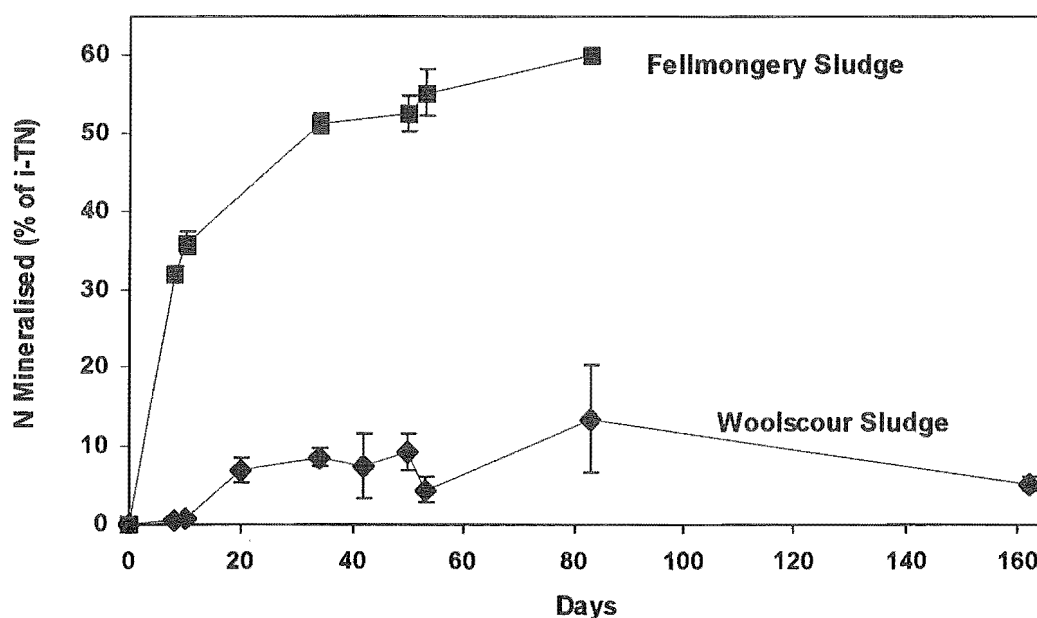


Figure 3.19. Nitrogen mineralisation during the decomposition of woolscour (◆) and fellmongery (■) sludges in sand-based microcosms. Incubation was at 22 °C and 20 % moisture. Means (\pm SE) are presented, $n = 2$ to 10.

3.4 CONSTRAINTS ON THE DECOMPOSITION OF WOOLSCOUR AND FELLMONGERY SLUDGES

3.4.1 N MINERALISATION VARIABILITY BETWEEN SUBSAMPLES OF WOOLSCOUR SLUDGE

During the decomposition of wooldscour sludge, there was temporal variation between the microcosms amended with sludges and incubated aerobic N mineralised at 20 % moisture and 22 °C of different N mineralisation experiments. This indicated that the wooldscour sludge was a highly variable substrate. This variability indicated that the absolute net-N mineralised during different treatments within an experiment could only be related to the within-experiment control and not between different experiments.

For example, in a given experiment (e.g. different temperatures (Figure 3.20)) wooldscour sludge mineralised 9 ± 1 % of the i-TN from the 22 °C and 20 % moisture incubated samples and 3 ± 0 % (i-TN) from the treatment at 5 °C. In a separate experiment (e.g. different moistures (Figure 3.21)) wooldscour sludge mineralised 5 ± 2 % of the i-TN from the 22 °C and 20 % moisture incubated samples and 3 ± 1 % of the i-TN from the manipulation at 5 % moisture. A wide range of N was mineralised from the wooldscour sludge incubated at 22 °C and 20 % moisture, 5 ± 2 % to 9 ± 1 i-TN, even though the individual standard errors of each experimental control were relatively small. Therefore given this variation, a *between*-experiment comparison of the absolute amounts of N mineralised at say 5 % moisture and at 5 °C is *not* a valid comparison. The magnitude of difference between the 22 °C and 20 % moisture incubated samples and the temperature or moisture manipulation *within* an experiment were not similar. For the temperature experiment there was 67 % less N mineralised at 5 °C than at 22 °C, where as in the moisture experiment there was 40 % less N mineralised at 5 °C than at 22 °C.

A conclusion from a between-experiment comparison for the above example would be “that net-N mineralisation during the decomposition of wooldscour sludge was negatively influenced by low temperature but not by low moisture”. Thus the absolute N mineralisation figures *are not compared between* experiments, but their magnitude is. This applied mainly to the wooldscour sludge, as the fellmongery sludge did not show the same degree of variability.

3.4.2 THE EFFECT OF TEMPERATURE ON N MINERALISATION

The aim of this experiment was to determine whether incubation temperature affected N mineralisation during decomposition of woolscur and fellmongery sludges. Net-N mineralisation for both sludges was strongly temperature dependent over the 50 days of decomposition (Figure 3.20). Woolscur sludge mineralised a maximum of 40 ± 2 % i-TN at 50 °C, which was the most N mineralised during any decomposition experiment for woolscur sludge throughout the entire research. Fellmongery sludge mineralised 30 ± 0 % i-TN at 10 °C, which was significantly less than the N mineralised at 22 °C (55 ± 1 % i-TN mineralised). There was *no* net N mineralisation from fellmongery sludge at, or above 43 °C (Figure 3.20).

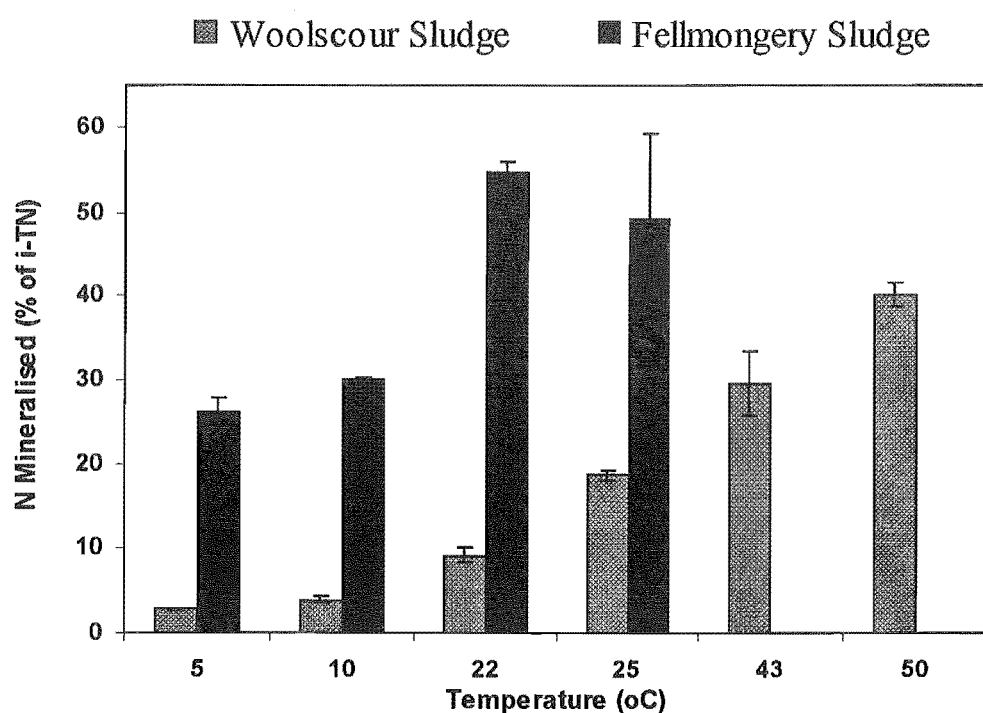


Figure 3.20. The effect of incubation temperature on N mineralisation from woolscur and fellmongery sludges. There was no N mineralised from fellmongery sludge at 43 or 50 °C. Microcosms were incubated at 20 % moisture for 50 days. Means (\pm SE), $n=3$.

3.4.3 THE EFFECT OF MOISTURE ON N MINERALISATION

The aim of this experiment was to determine whether the level of moisture in microcosms affected N mineralisation during decomposition of woolsour and fellmongery sludges. The N mineralised during the decomposition of woolsour and fellmongery sludges was not affected by moisture ranging from 10 % to 35 % (Figure 3.21). After 50 days decomposition, woolsour sludge mineralised <5 % i-TN irrespective of the moisture level. The amount of N mineralisation was low even for woolsour sludge. Fellmongery sludge, however, mineralised significantly less N at 5 % moisture (42 ± 4 % i-TN mineralised), than at the 10 % (or higher) moisture level (56 ± 1 % i-TN mineralised).

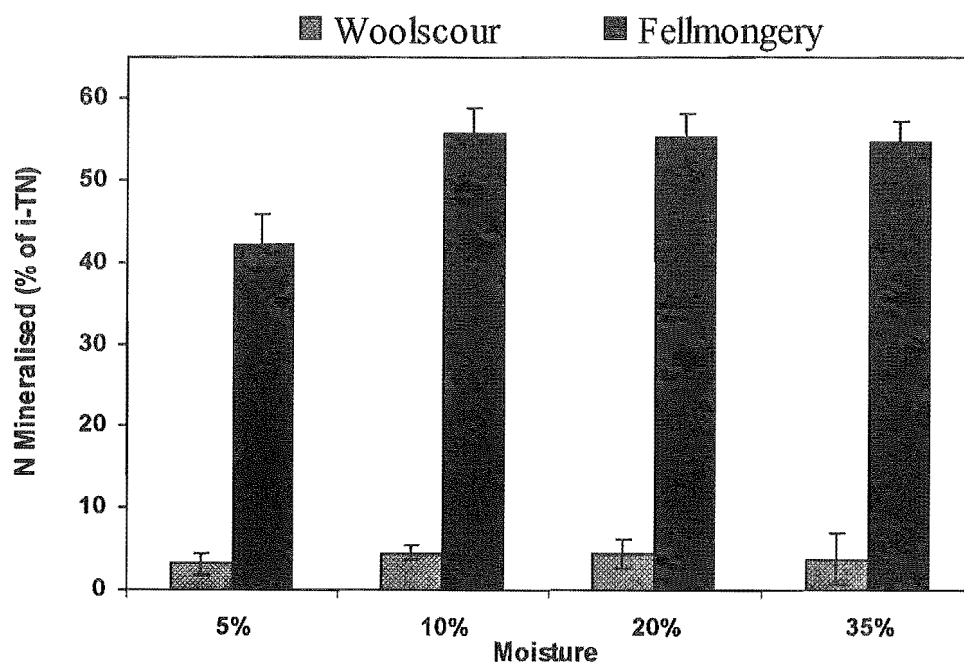


Figure 3.21. The effect of moisture on N mineralisation from woolsour and fellmongery sludges. Incubation was at 22 °C for 50 days. Means are presented (\pm SE), $n=3$.

3.4.4 THE EFFECT OF ANAEROBIC CONDITIONS ON N MINERALISATION

The aim of this experiment was to determine whether an anaerobic atmosphere affected N mineralisation during decomposition of woolscur and fellmongery sludges. Anaerobic conditions (Section 2.4.8) affected the amount of N mineralised from fellmongery sludge to a greater extent than they did with woolscur sludge. Fellmongery sludge mineralised 15 ± 1 % i-TN and woolscur sludge mineralised 5 ± 0 % i-TN, after 108 days under anaerobic conditions at 22 °C and 30 % moisture (Figure 1.1).

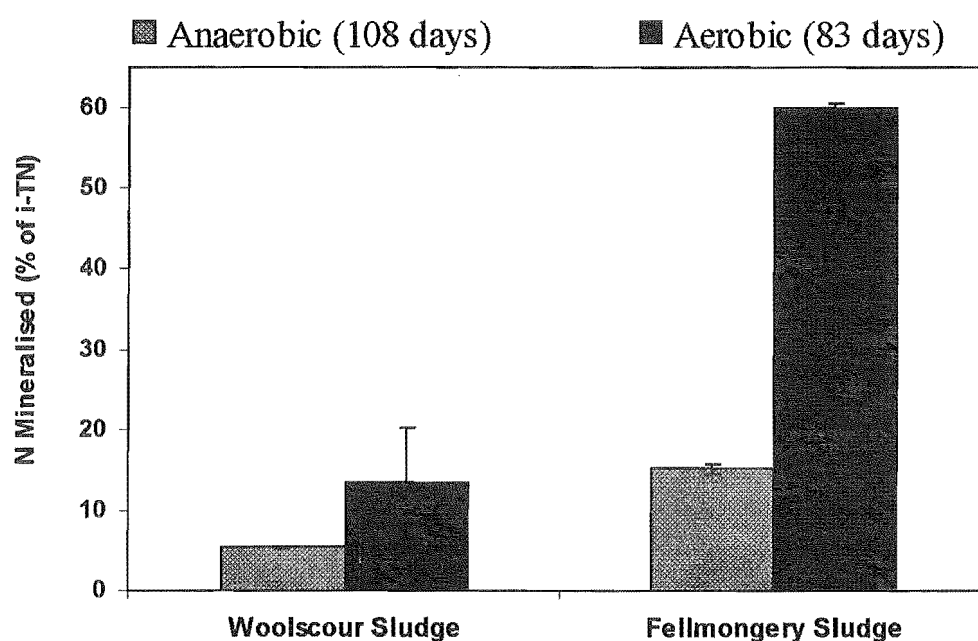


Figure 3.22. The effect of anaerobic incubation conditions on N mineralisation from woolscur and fellmongery sludges. Incubation was at 22 °C and 40 % moisture for 108 days for anaerobic conditions and for 83 days for aerobic conditions. Means are presented (\pm SE), $n=3$.

3.4.5 THE EFFECT OF DIFFERENT MATRICES (SAND AND SOIL) ON N MINERALISATION

Sand and soil were used in mineralisation microcosms as matrices to minimise osmotic effects of metabolite concentration. However soil, unlike ignited sand, also has the potential to mineralise N (and C), which can make interpretation of net N mineralisation more difficult (Section 4.2.4). The aim of this experiment was to determine whether the matrix used in the microcosms affected N mineralisation during decomposition of woolscur and fellmongery sludges.

The decomposition of woolscur and fellmongery sludges in a sand or soil matrix did not greatly influence N mineralisation over an incubation period of 30 to 50 days. However, there was a trend that more N was mineralised from decomposing woolscur sludge in soil than in sand, and the opposite trend for fellmongery sludge.

After 30 days of decomposition, woolscur sludge had mineralised 9 ± 2 % of i-TN in a sand matrix and 15 ± 3 % of i-TN in a soil matrix (Figure 3.23 (a)). After 50 days of decomposition, woolscur sludge had mineralised 9 ± 2 % of i-TN in a sand matrix and 11 ± 3 % of i-TN in a soil matrix (Figure 3.23 (b)). After 30 days of decomposition, fellmongery sludge had mineralised 51 ± 1 % of i-TN in a sand matrix and 46 ± 1 % of i-TN in a soil matrix (Figure 3.23 (a)). After 50 days of decomposition, fellmongery sludge had mineralised 52 ± 2 % of i-TN in a sand matrix and 45 ± 4 % of i-TN in a soil matrix (Figure 3.23 (b)).

3.4.6 NUTRIENT CYCLING AND WOOLSCOUR AND FELLMONGERY SLUDGES

The results so far have shown that woolscur and fellmongery sludges decompose and release mineral N into the surrounding environment, although to different extents. The aim of the next series of experiments was to determine the effect that woolscur and fellmongery sludges have on the release of mineral N when decomposed in the presence of other substrates, i.e. their influence on nutrient cycling. Three experiments were used to address this question.

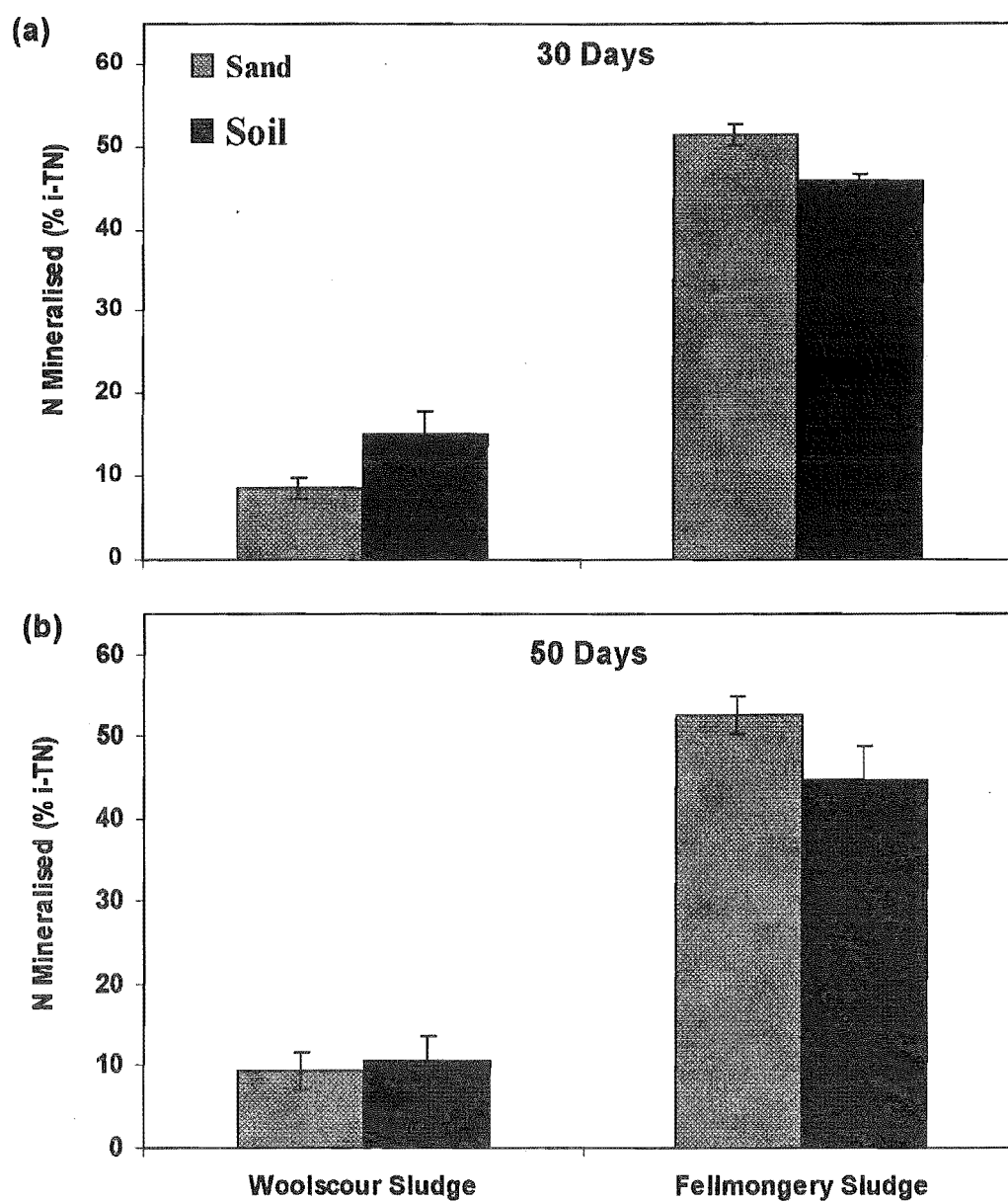


Figure 3.23. The effect of sand and soil matrices on N mineralisation during the decomposition of woalscour and fellmongery sludges in (a) after 30 days decomposition and (b) after 50 days decomposition. Incubation was at 22 °C and 20 % moisture. Means (\pm SE), $n = 3$.

(i) The co-incubation of woollscour and fellmongery sludges, which was used to determine the net effect of a readily decomposable substrate on the net-N mineralised when in the presence of a more recalcitrant one.

(ii) The co-incubation of woollscour sludge with flower petals and of fellmongery sludge with flower petals, which was used to gauge the influence of the sludges on nutrient cycling from plant material.

(iii) The decomposition of the sludges and samples of wool in soil that had previously received amendment with woollscour and fellmongery sludges.

After 50 days of decomposition, the co-incubation of woollscour and fellmongery sludges in a sand matrix showed that woollscour sludge had mineralised 6 ± 2 % (i-TN) and the fellmongery sludge 56 ± 2 % of i-TN (Figure 3.24). There was a weak trend that less N was mineralised when the sludges were mixed together than expected based on the N mineralised by each sludge individually. For example, when the i-TN in a microcosm was 23 % woollscour sludge-N and fellmongery sludge-N 77 % (23-77 in Figure 3.24), 38 ± 4 % (i-TN) was mineralised experimentally, whereas 44 % (i-TN) was expected to be mineralised (Figure 3.24).

After 30 days of decomposition, *Genista* sp. and *Sophora tetraptera* (Kowhai) petals had mineralised only 1 ± 0 % and 6 ± 3 % (i-TN), respectively (Figure 3.25). There was minimal difference in the N mineralised when the petals were decomposed in the presence of either sludge, although there was a weak trend of decreased mineralisation when petals were mixed with fellmongery sludge. In theory (based on N mineralised by the sludge unamended), Kowhai petals mixed with fellmongery sludge were expected to mineralise 29 % (i-TN), however experimentally 24 ± 7 % (i-TN) was mineralised. In theory, *Genista* petals were expected to mineralise 25 % (i-TN), while 21 ± 2 % (i-TN) was mineralised experimentally. During the co-incubation of woollscour sludge and Kowhai petals, in theory 8 % (i-TN) was expected to be mineralised, whereas experimentally 11 ± 2 % (i-TN) was mineralised. However, when, woollscour sludge and *Genista* petals were co-incubated, in theory 5 % (i-TN) was expected to be mineralised, whereas 2 ± 2 % (i-TN) was mineralised (Figure 3.25).

After 30 days of decomposition, raw wool mineralised 32 ± 5 % (i-TN) in the unamended control soil, which dropped to only 16 ± 7 % when decomposed in the

woolscour sludge-amended soil (Figure 3.26). Woolscour sludge mineralised 20 ± 3 % (i-TN) in the control soil, which also dropped when decomposed in the woolscour sludge-amended soil (13 ± 3 % i-TN mineralised). However, wool that had been scoured (all the grease, suint and dirt removed) showed an increase in net-N mineralisation when decomposition was in the woolscour sludge-amended soil (17 ± 5 % i-TN mineralised) compared to 12 ± 2 % (i-TN) mineralised in the unamended control soil (Figure 3.26). The fellmongery sludge mineralised 47 ± 1 % (i-TN) in the control soil, which decreased to 28 ± 2 % (i-TN) when decomposed in fellmongery sludge-amended soil (Figure 3.26).

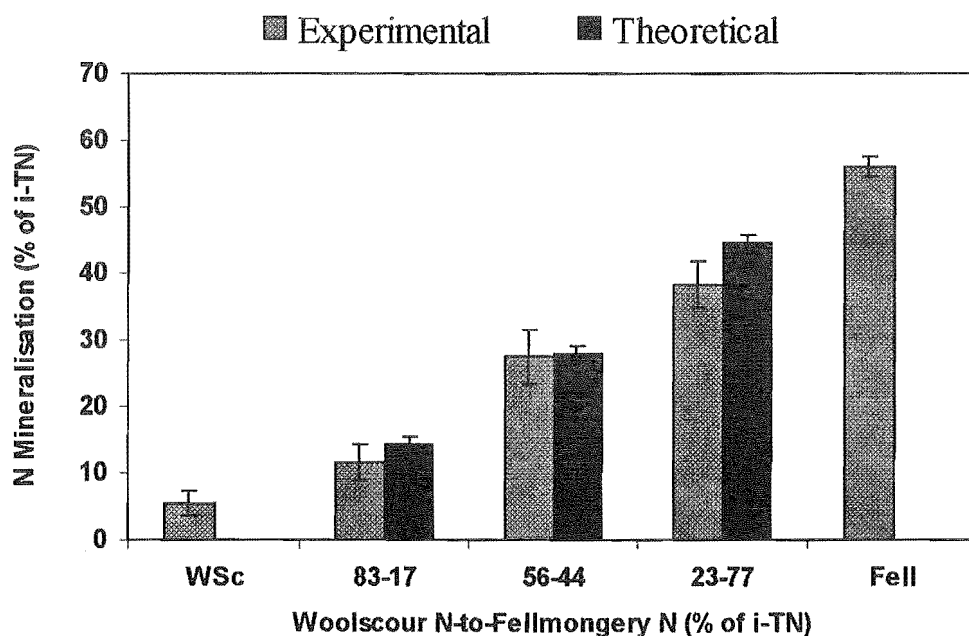


Figure 3.24. Co-incubation of woolscour (WSc) and fellmongery (Fell) sludges. Incubation was for 50 days at 22 °C and 20 % moisture. Means (\pm SE), $n=3$.

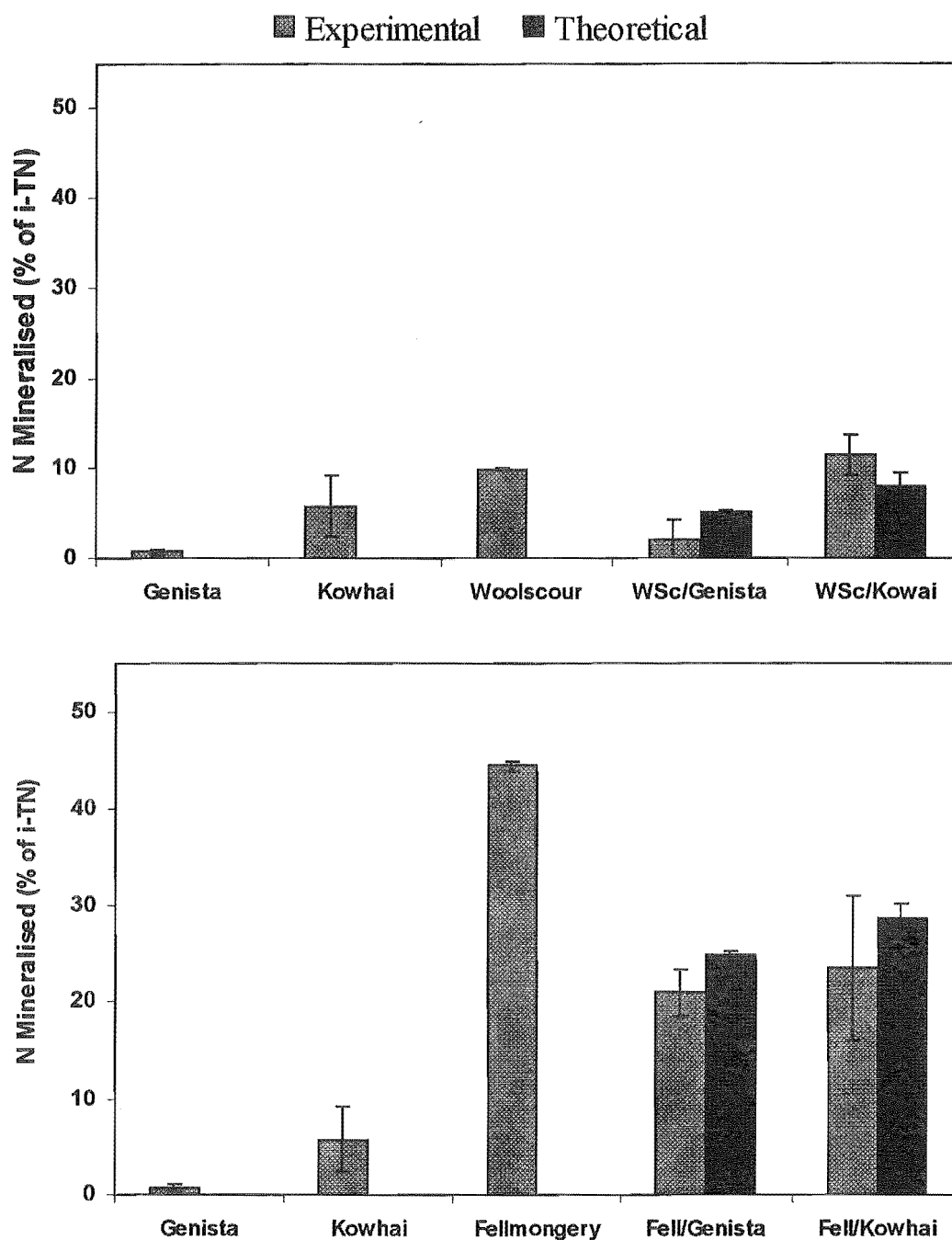


Figure 3.25. Co-incubation of (a) Wooldscour sludge (WSc) with the petals of *Genista* sp. and *Sophora tetraptera* (Kowhai) and (b) fellmongery (Fell) sludge with the petals of *Genista* sp. and *S. tetraptera* (Kowhai). The petals and sludge were mixed 50-50 on N basis (w/w N). Incubation was for 30 days at 22 °C and 20 % moisture. Means (\pm SE), n=3.

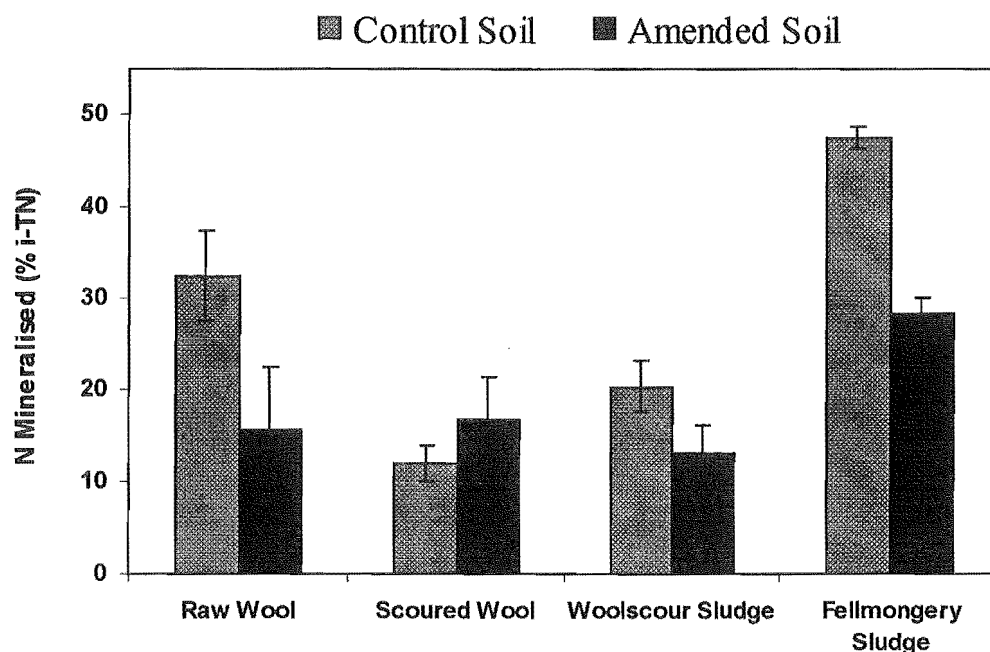


Figure 3.26. The N mineralised during the decomposition of raw wool, scoured wool and woollscour sludge in soil-based microcosms. The soil used was obtained from field soil amended with woollscour sludge (at 60 kg N ha⁻¹) 10 months previously. The decomposition of fellmongery sludge in soil-based microcosms; the soil used was obtained from field soil amended with fellmongery sludge (at 195 kg N ha⁻¹) 10 months previously. Incubation was for 30 days at 22 °C and 20 % moisture. Means (\pm SE), n=3.

3.5 CONSTRAINTS ON WOOLSCOUR SLUDGE DECOMPOSITION

3.5.1 THE EFFECT OF SOIL INOCULUM FROM SOIL TREATED LONG-TERM WITH WOOLSCOUR WASTES

Soil receiving organic wastes for a long period of time is likely to become enriched with organisms capable of decomposing the specific wastes. The aim of this experiment was to determine whether the N mineralised during the decomposition of woollscour sludge was affected when the inoculum used (in sand-based microcosms) had been extracted from soil that had received woollscour wastes for over 12 years.

The organisms extracted from woollscour waste-amended soil did not promote the mineralisation of N (Table 3.14). This was consistent over long-term incubation of 162 days. This implied that the woollscour waste-amended soil was not enriched with organisms specialised for woollscour waste decomposition.

Table 3.14. The N mineralised during decomposition of woolscour sludge in sand-based microcosms where the inoculum added came either from soil that had never received woolscour wastes (untreated inoculum) or from soil that had received woolscour wastes for >12 years (treated inoculum). Means (\pm SE), $n=3$.

Days	Untreated Inoculum	Treated Inoculum
	N mineralised (% of i-TN)	
50	9.39 (\pm 2.31)	8.85 (\pm 0.83)
90	12.43 (\pm 0.68)	13.65 (\pm 1.02)
162	5.23 (\pm 0.00)	5.27 (\pm 1.54)

3.5.2 THE EFFECT OF WOOLGREASE ON PROTEIN DECOMPOSITION

The aim of this experiment was to determine whether the low amount of N mineralised from woolscour sludge was due to the woolgrease associated with the sludge. This was addressed by mixing casein with woolgrease. During 116 days of decomposition, the casein was readily decomposed and mineralised 68 ± 3 % (i-TN; Figure 3.27). When woolgrease was mixed with casein at 15 % (woolgrease-casein w/w) the mineralised N increased to 96 ± 18 % (i-TN). When the mixture was 50 % woolscour-casein (w/w), 40 ± 5 % (i-TN) was mineralised (Figure 3.27). The level of grease associated with woolscour sludge was 33 % (Table 3.9).

3.5.3 THE DECOMPOSITION OF WOOL

Scanning electron micrographs (SEM) showed that, prior to decomposition, the fibres of woolscour sludge were covered with a continuous layer of grease and there were few microbes associated with the sludge fibres (Figure 3.29 (a)). After 26 days decomposition (at 22 °C and 20 % moisture in a sand matrix), SEM micrographs indicated that there had been only a slight increase in the number of microbes present (Figure 3.29 (b)). After 36 days of decomposition, the SEM examination showed that

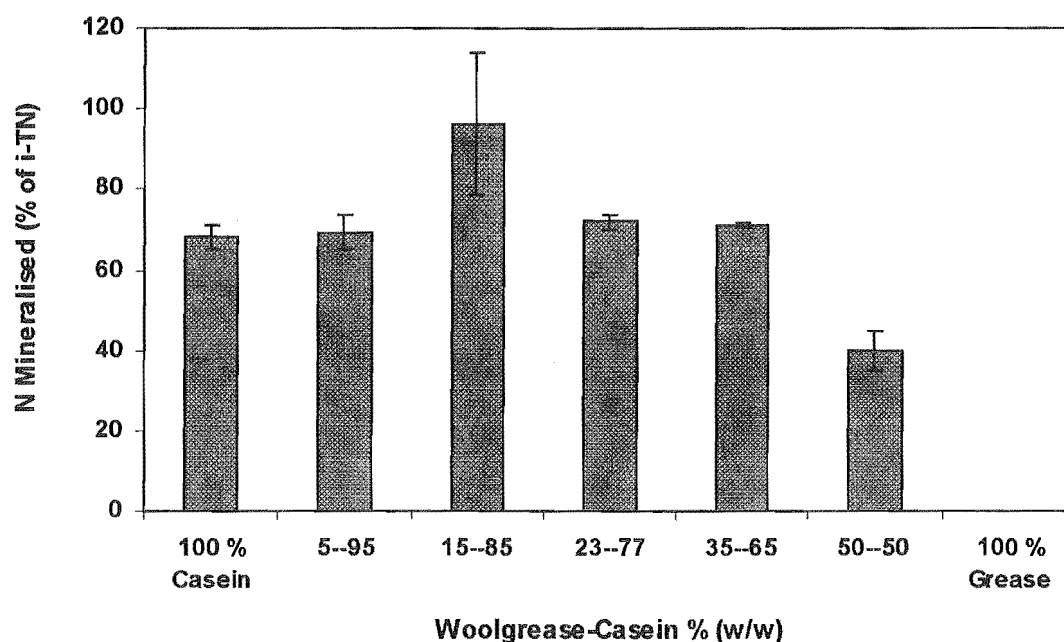


Figure 3.27. Nitrogen mineralised during the decomposition of casein mixed with woolgrease at different ratios of woolgrease:casein. Incubation was for 116 days at 22 °C and 20 % moisture. Means (\pm SE), $n=2$.

the decomposing wooldour sludge had what appeared to be slime or mucilage over much of the sludge fibres, with established fungal hyphae (Figure 3.29 (c)).

To gain a better understanding of how the wool fibre portion of wooldour sludge may decompose, two samples of wool were used: raw and scoured wool. Raw wool was used as wool fibre in its natural state, *i.e.* covered by woolgrease with the associated suint, dirt and moisture (Figure 3.30 (a)) and represented a model for the continuous layer of woolgrease associated with wooldour sludge. Wool, scoured by the mini bowl system (Figure 1.3), was used to represent wool fibre without dirt, grease or suint (Figure 3.30 (b)). The raw and scoured wool had similar amounts of TN, which were three-times that of the wooldour sludge and its fibre-dirt fraction (Table 3.15). Unlike wooldour sludge and its fibre-dirt fraction, raw and scoured wools had very little mineral N associated with them (Table 3.15). The fibre-dirt fraction of wooldour sludge is shown in Figure 3.30 (c) and looks similar to wool fibres that had been scoured.

During 30 days of decomposition in soil-based microcosms, 32 ± 5 % (i-TN) of raw wool was mineralised and 12 ± 2 % (i-TN) of the scoured wool was mineralised (control soils in Figure 3.26). During decomposition, the fibre-dirt fraction of

woolscour sludge mineralised 27 ± 4 % of i-TN in 50 days, which was significantly more than that mineralised from woolscour sludge (9 ± 2 % i-TN; Figure 3.28). For the fibre-dirt fraction after 162 days there was no replication due to the duplicate being broken (Figure 3.28).

Table 3.15. Total N and the proportion of total N as mineral N in the different types of wool used for N mineralisation experiments.

Sample	Organic Matter	Total Nitrogen		Mineral N	
		(dw)	(ash-free)	(dw)	(ash-free)
		(%)		(% of TN)	
Woolscour Sludge	62	4.47	7.2	4.0	2.4
Fibre-Dirt Fraction	44	4.70	10.8	3.0	1.3
Raw Wool	96	14.42	15.0	0.3	0.3
Scoured Wool	97	14.76	15.3	0.4	0.4

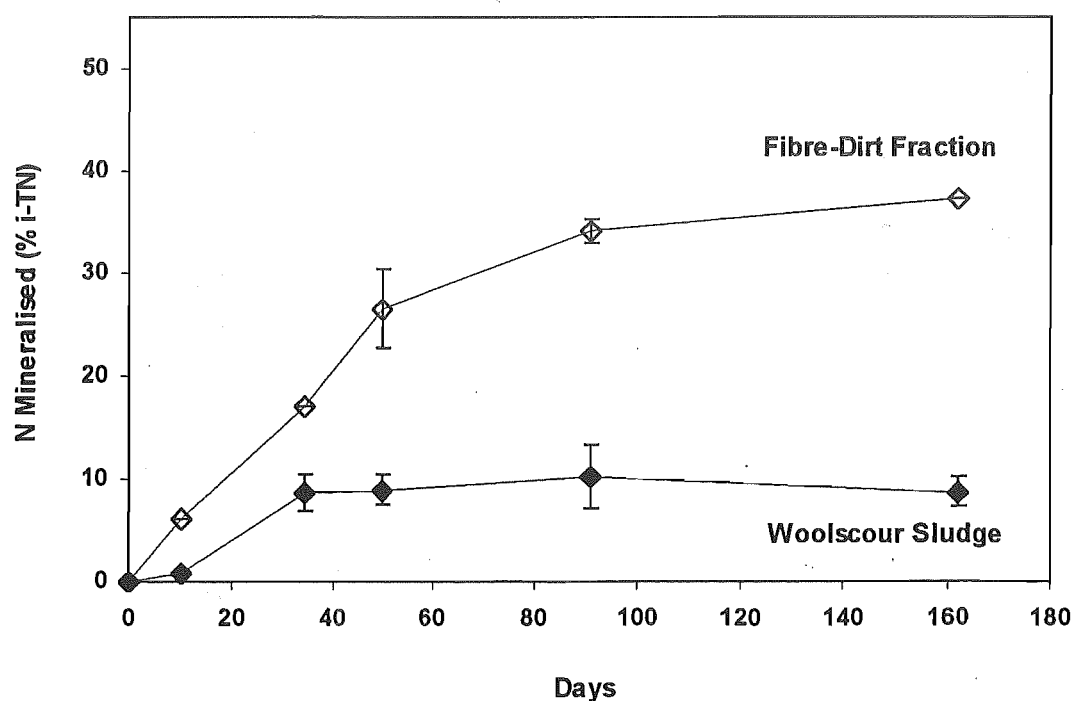


Figure 3.28. Nitrogen mineralised during the decomposition of woolscour sludge (◆) and the fibre-dirt fraction (◇) of woolscour sludge in sand-based microcosms. Microcosms were at 22 °C and 20 % moisture. Means are presented (\pm SE), n=2.

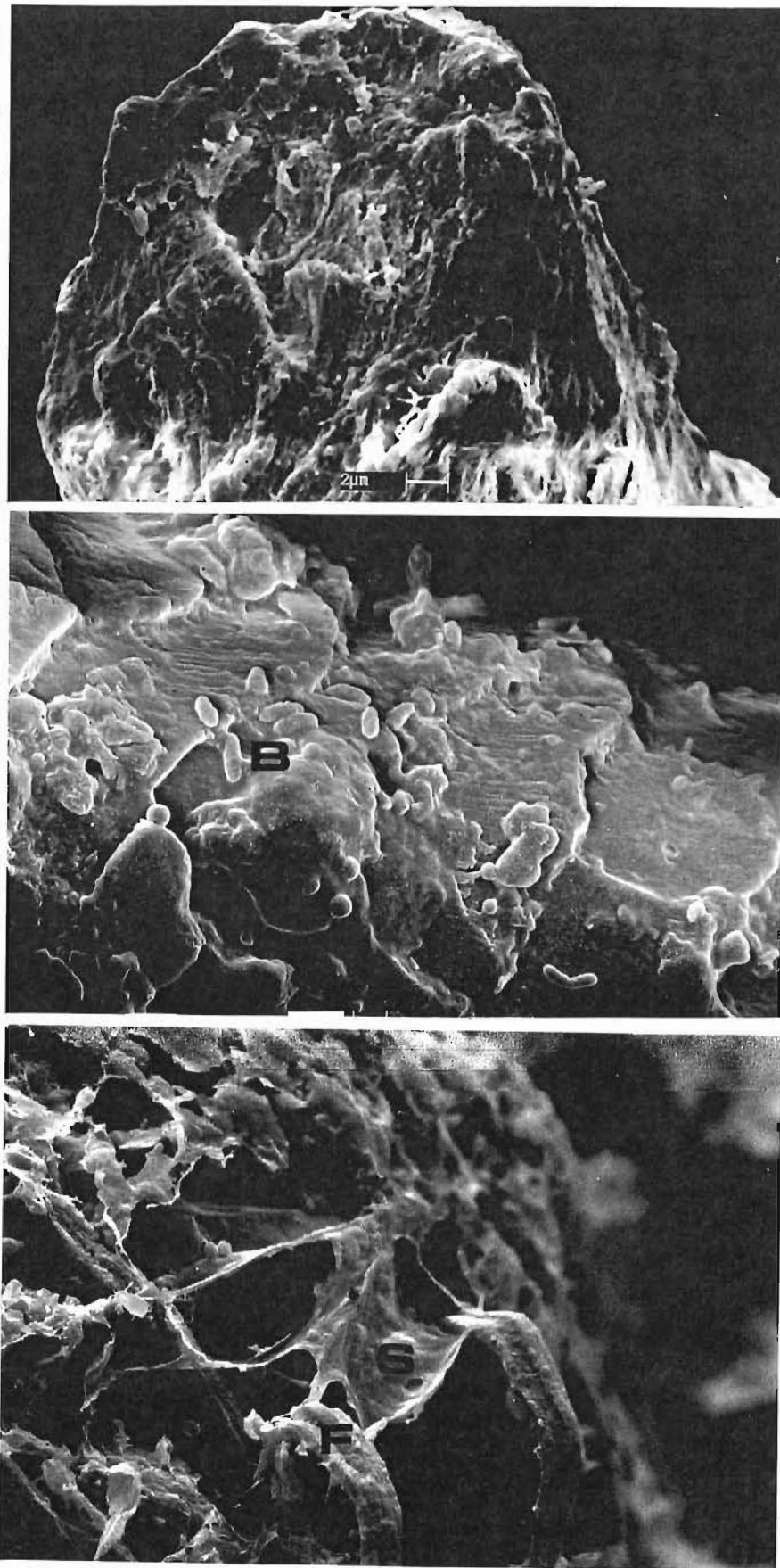


Figure 3.29. Scanning electron micrograph of woolscour sludge, (a) showing the continuous layer of woolscour grease over the wool fibre (5,000x), (b) after 26 days decomposition (5,000x) and (c) after 36 days decomposition (5,000x). B = bacterial cells, F = fungal hyphae, and S = microbial slime or mucilage.

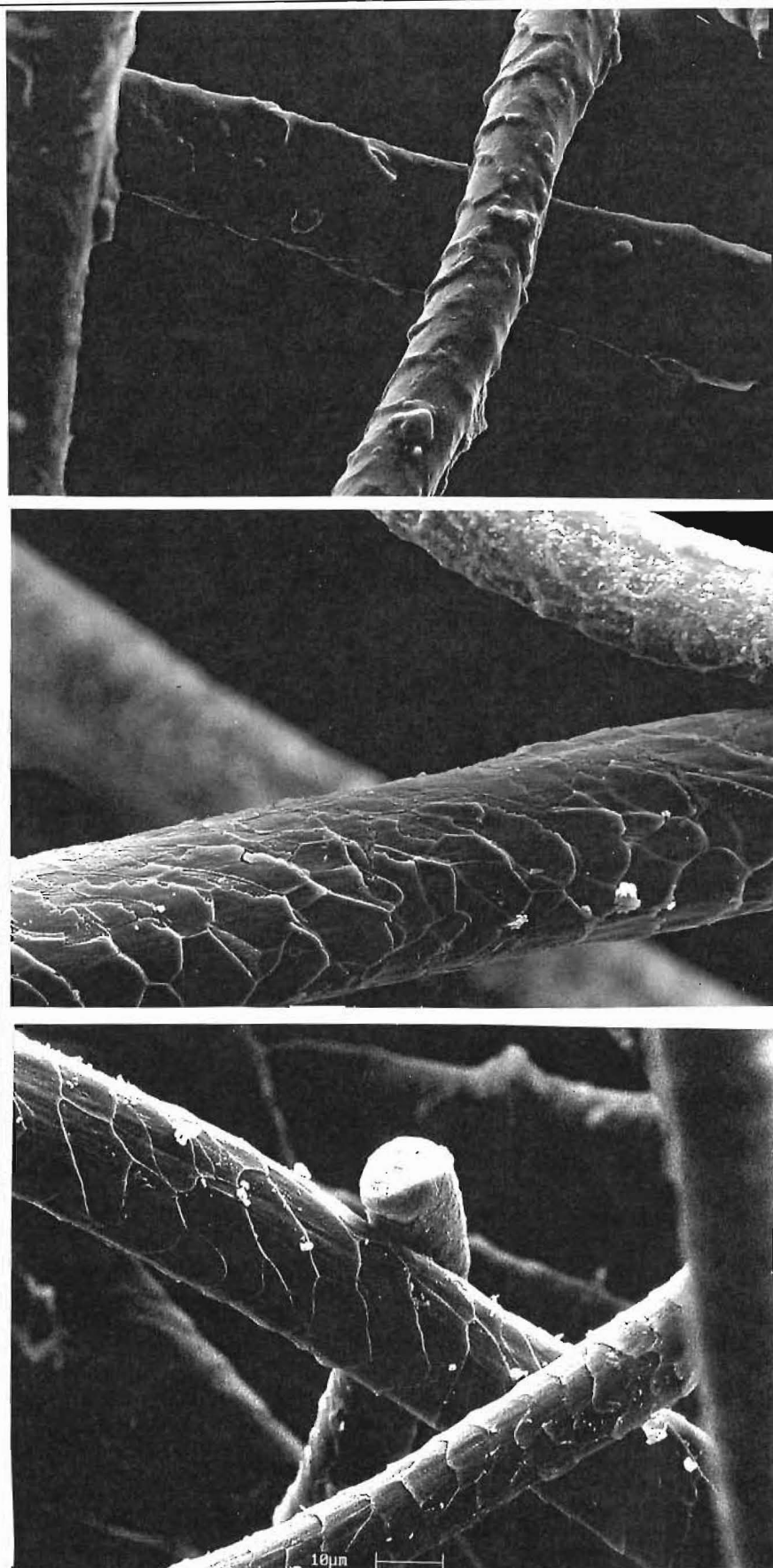


Figure 3.30. Scanning electron micrographs (x1000) of (a) raw wool (b) scoured wool and (c) the fibre-dirt fraction of woolscour sludge.

3.5.4 THE EFFECT OF GLUCOSE AMENDMENT ON WOOLSCOUR SLUDGE DECOMPOSITION

The aim of this experiment was to determine whether the decomposition of wooldscour sludge was partially limited by readily available C. To address this question, glucose was added to the wooldscour sludge either immediately or after some sludge decomposition had occurred (Section 2.6.4). Only when glucose was added early, was more N mineralised from wooldscour sludge (Figure 3.31). After 50 days of wooldscour sludge decomposition, non-amended wooldscour sludge mineralised 15 ± 5 % i-TN, which increased to 23 ± 1 % i-TN when amended with 1 % glucose (w/w; Figure 3.31 (a)). When wooldscour sludge was amended with glucose at 10 % (w/w), 15 ± 3 % i-TN was mineralised (Figure 3.31 (a)).

After 60 days of wooldscour sludge decomposition the non-amended wooldscour sludge had mineralised 7 ± 7 % of i-TN (Figure 3.31 (b)). This was similar to the N mineralised when glucose had been added to the sludge during decomposition. For wooldscour sludge amended with 8 % glucose after 30 days of decomposition, 6 ± 1 % of i-TN was mineralised and when the sludge was amended at 23 % glucose during decomposition, 5 ± 3 % of i-TN was mineralised.

3.5.5 THE EFFECT OF AMMONIUM NITRATE AMENDMENT ON WOOLSCOUR SLUDGE DECOMPOSITION

The aim of this experiment was to determine whether the N mineralised during wooldscour sludge decomposition was limited by the amount of available N initially present in the system. To address this question wooldscour sludge (W) was amended with ammonium nitrate (AN) during the setting-up of the microcosms. Wooldscour sludge and ammonium nitrate contributed to the ~10 mg of N per microcosm. The mineral N present at day zero was subtracted from the mineral N present after 34 and 53 days. It was, therefore, assumed that the N mineralised during sludge decomposition originated solely from the wooldscour sludge. This assumption was not checked, as labelled ammonium nitrate was not available.

After 34 days of decomposition, wooldscour sludge amended with ammonium nitrate at 88 W-12 AN (w/w) mineralised 9 ± 1 % of wooldscour-N, which was less than

the 24 % of wooldscour-N that was predicted to be mineralised (Figure 3.32 (a)). As the amount of inorganic N added to the microcosms increased, the wooldscour sludge-N mineralised approached the theoretical mineralisation; 7 ± 2 % of wooldscour-N was mineralised at 31 W-69 AN (w/w), *c.f.* 8 % of wooldscour-N that was predicted to be mineralised (Figure 3.32 (a)).

After 53 days decomposition, there was essentially no difference in the N mineralised experimentally and that predicted (Figure 3.32 (b)). At the lowest amendment rate (88 W-12 AN (w/w)) 20 ± 4 % of wooldscour-N was mineralised *c.f.* 20 % in theory. At the highest inorganic amendment 8 ± 1 % of wooldscour-N was mineralised *c.f.* 7 % in theory (Figure 3.32 (b)).

3.5.6 THE EFFECT OF TRACE METALS AMENDMENT ON WOOLSCOUR SLUDGE DECOMPOSITION

An observation during the temperature profile experiment (Section 3.4.2) suggested that iron-requiring organisms were involved in the decomposition of wooldscour sludge. In some microcosms incubated at 30 and 43 °C rust particles had dripped from some of the wires suspending the acid traps in the microcosms. Wherever rust had dripped there was an abundance of fungal growth, often filling the microcosm space, and was well in excess of any other fungal growth observed on wooldscour sludge for any experiment. This suggested enhancement of microbial growth was due to the presence of iron. Therefore, the aim of this experiment was to determine whether more N was mineralised during wooldscour sludge decomposition when Fe(II) was present. To address this question, wooldscour sludge was amended with Fe(II) either individually or as a component of a trace metal solution.

After 86 days decomposition, wooldscour sludge amended with Fe(II) either individually or as part of a trace metals solution, up to and including 870 ppm Fe(II), showed increased N mineralisation compared to the unamended control (Figure 3.33). The strongest enhancement of mineralisation occurred when Fe(II) was added individually at 87 ppm (20 ± 3 % i-TN mineralised *c.f.* 13 ± 7 % i-TN mineralised from the unamended control).

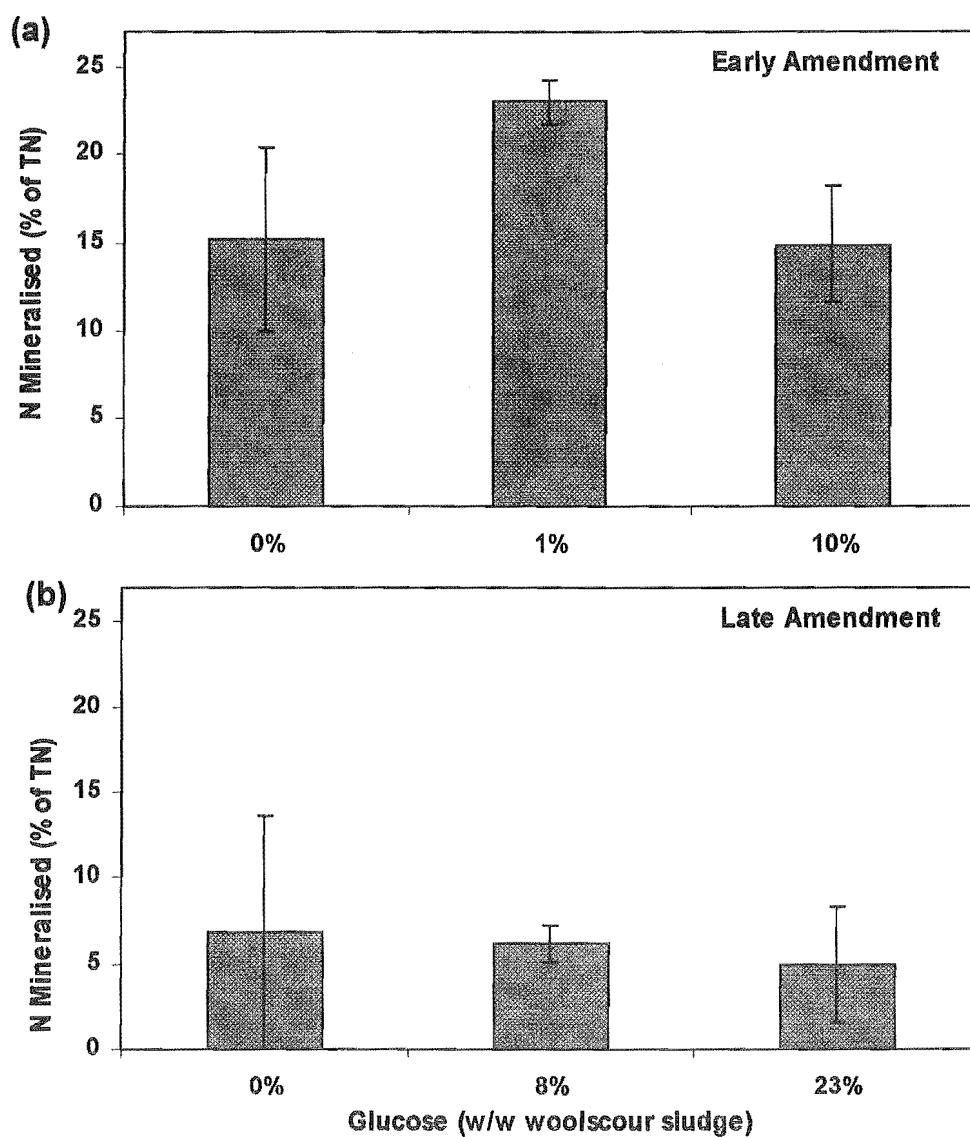


Figure 3.31. Nitrogen mineralised during the decomposition of woolscour sludge amended with glucose. (a) Early amendment, incubation for 50 days, and (b) after 30 decomposition days (late amendment) and microcosms were incubated for a further 30 days. Means (\pm SE), $n=3$.

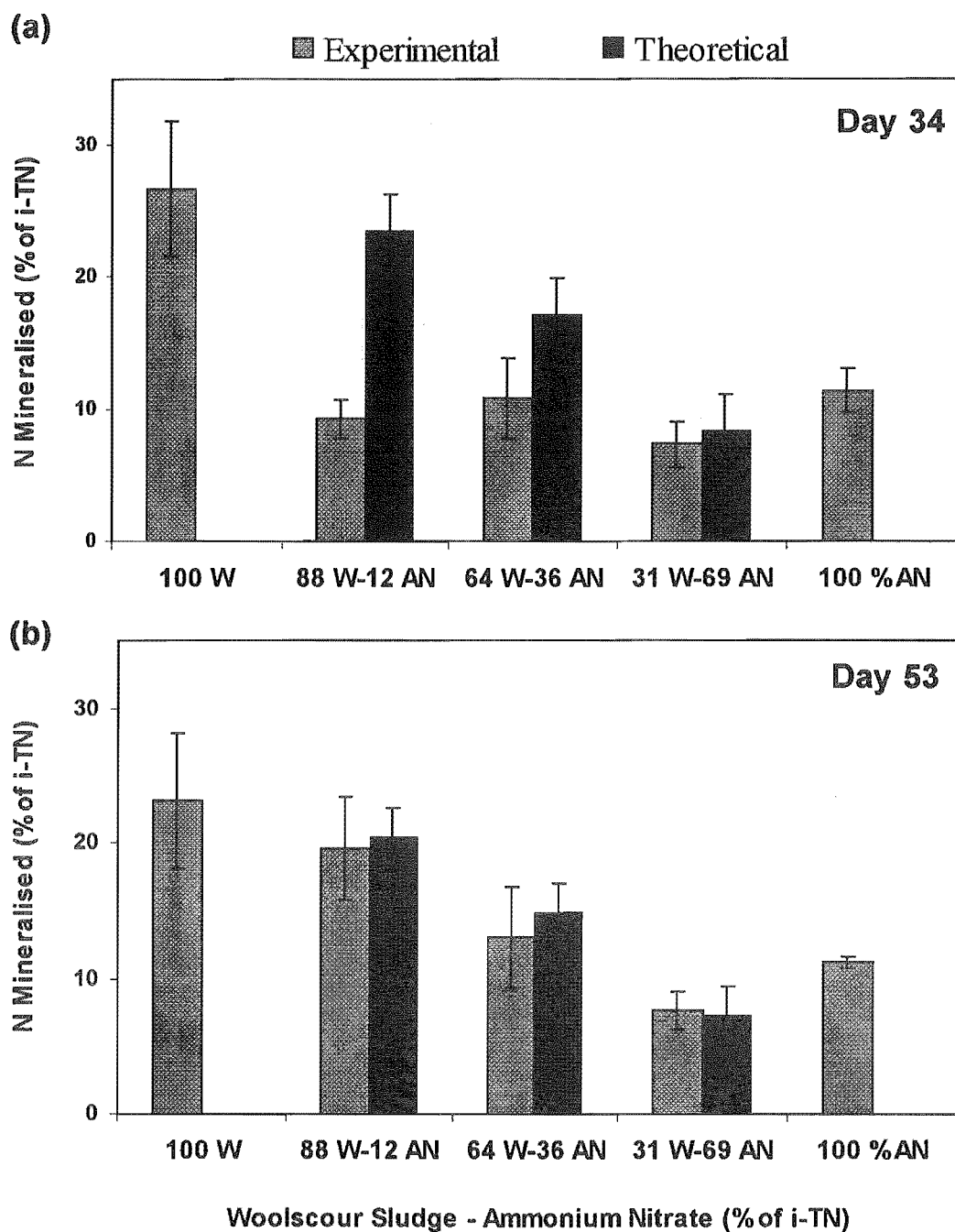


Figure 3.32. Nitrogen mineralised during the decomposition of woolscour sludge (W) amended with ammonium nitrate (AN). (a) Incubation was for 34 days and (b) incubation for 53 days. Incubation was at 22 °C and 20 % moisture. Means (\pm SE), $n=3$.

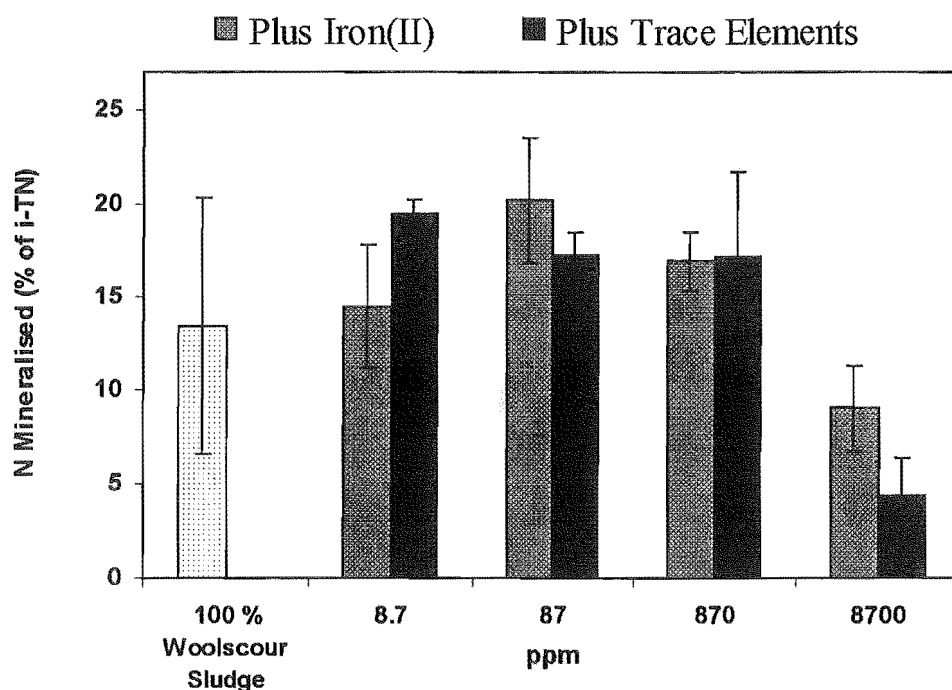


Figure 3.33. Nitrogen mineralised during the decomposition of woolsour sludge amended with Fe (II), either as FeCl_2 or as a component of a trace metal solution (Table 2.7). Incubation was at 22 °C and 20 % moisture for 86 days. Means (\pm SE), $n=3$.

3.6 CONSEQUENCES OF FELLMONGERY SLUDGE DECOMPOSITION

Organic substrates that are high in N have the potential to leach mineral N, especially nitrate, during decomposition. The aim of this set of experiments was to determine whether a consequence of the extensive decomposition seen for fellmongery sludge was the leaching of mineral N, in particular nitrate (Figure 3.34).

3.6.1 DECOMPOSITION ON A SAND MATRIX (LEACH-1 TO LEACH-3)

Experiments Leach-1 and Leach-2 addressed the question whether decomposing fellmongery sludge had the potential to leach mineral N, including nitrate. The two experiments differed only in the frequency of leaching (and hence volume of water added); Leach-1 was irregularly leached and received 760 mL of water, while Leach-2 was regularly leached and received 1460 mL of water (Table 2.8). Both regimes leached similar amounts of mineral N, with 30 ± 0 % of i-TN leached as mineral-N

from Leach-1 and 29 ± 1 % i-TN from Leach-2 (Table 3.16 and Figure 3.35 (a, b)). Leach-2 produced less nitrate (20 ± 3 % of applied N) than Leach-1 (26 ± 1 % of applied N), however the difference was not significant $p = 0.1110$ (Figure 3.35 (a, b)). For both experiments, nitrate was the principal mineral N species leached (Table 3.16, Figure 3.34 and Figure 3.35).

Experiment Leach-3 addressed the question whether the presence of grass would reduce the amount of mineral N and of nitrate leached during fellmongery sludge decomposition. After 99 days of fellmongery sludge decomposition and leaching, 25 ± 1 % of applied N had been leached as mineral N, with 18 ± 1 % of applied N leached as nitrate-N (Figure 3.35 (c)). Significantly less nitrate was leached from Leach-3 than that leached from Leach-1 ($p = 0.0014$), but not when Leach-3 was compared with Leach-2 ($p = 0.5945$). Less ammonium-N was leached from Leach-3 (7 ± 1 % of applied N) than from Leach-2 (9 ± 3 % of applied N), however the difference was not significant ($p = 0.4265$; Figure 3.35). These results indicated that the presence of ryegrass reduced the amount of ammonium N leached when water was applied to the columns frequently, suggesting that the grass had taken up ammonium-N rather than nitrate-N.

Table 3.16. The average mineral-N and nitrate-N leached from fellmongery sludge-amended leaching columns (at a rate equivalent to 350 kg N ha^{-1}). Means (\pm SE), $n=3$, corrected for mineral N leached from control columns.

Treatment	Cumulative Mineral-N Leached	Cumulative Nitrate-N Leached	
	(% applied N \pm SE)	(% applied N \pm SE)	(% min-N leached)
Leach-1 (sand, irregular leaching)	29.8 ± 0.4	26.0 ± 0.6	87
Leach-2 (sand, regular leaching)	29.1 ± 0.7	20.0 ± 2.9	68
Leach-3 (sand, regular leaching, ryegrass)	24.8 ± 1.0	18.3 ± 0.8	74
Leach-4 (soil, regular leaching)	39.1 ± 0.8	36.3 ± 2.0	93

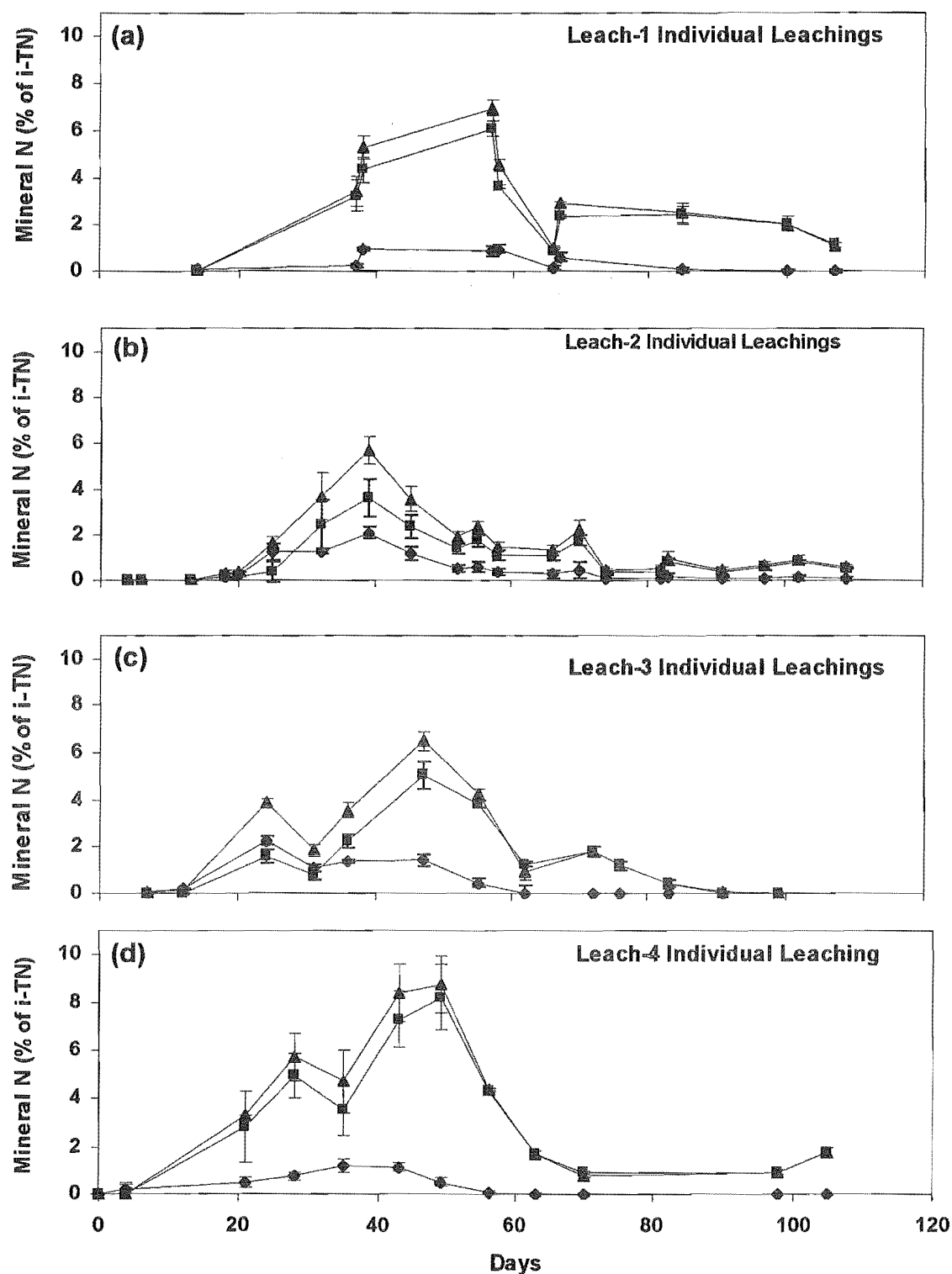


Figure 3.34. Mineral-N (▲), nitrate-N (■) and ammonium-N (◆) leached, on a daily basis, during the decomposition of surface-applied fellmongery sludge (at a rate equivalent to 350 kg N ha^{-1}). (a) Leach-1, (b) Leach-2, (c) Leach-3, and (d) Leach-4. Incubation was at 22°C . Mean ($\pm \text{SE}$), $n=3$, corrected for mineral N leached from control columns.

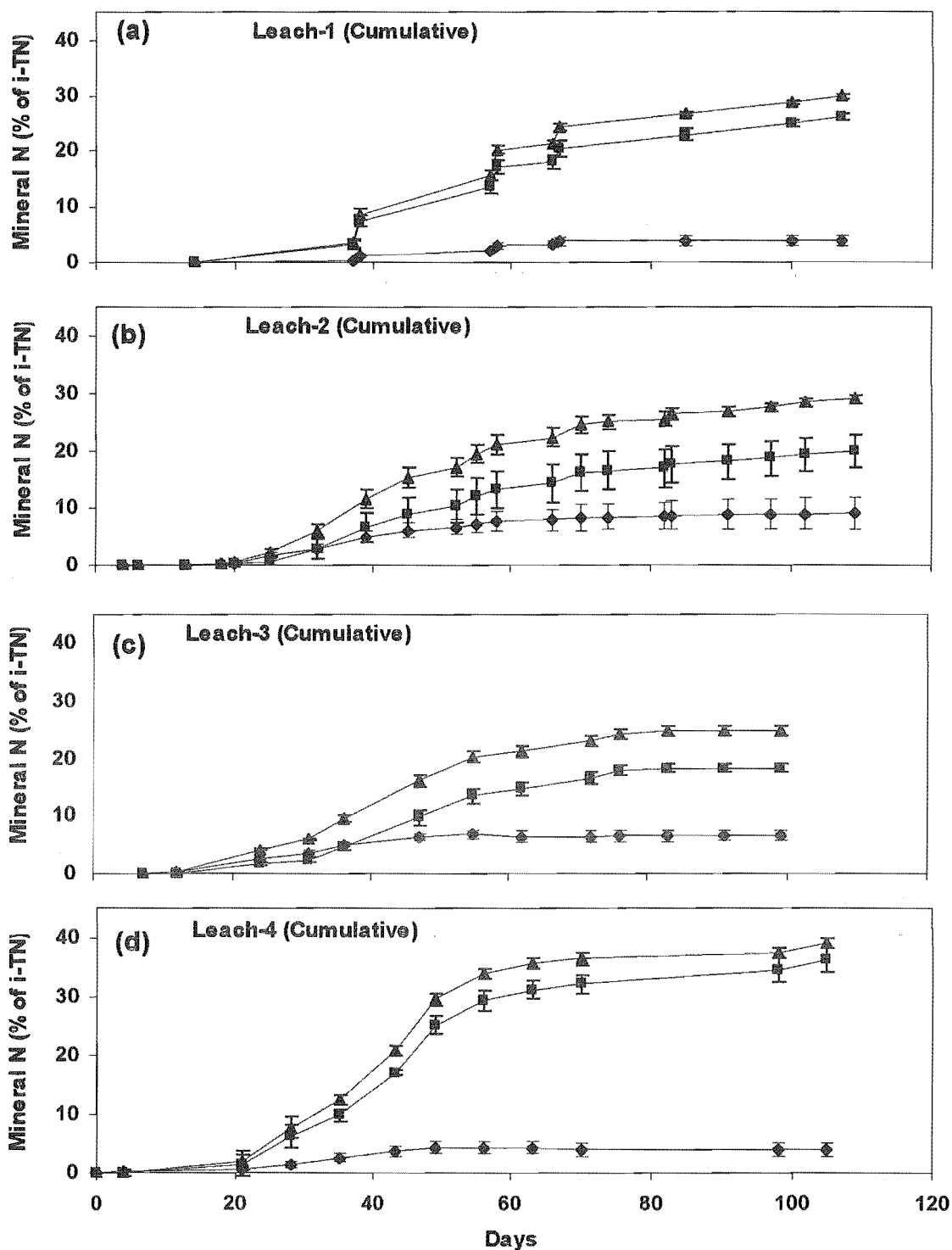


Figure 3.35. Mineral-N (\blacktriangle), nitrate-N (\blacksquare) and ammonium-N (\blacklozenge) leached, on a cumulative basis, during the decomposition of surface-applied fellmongery sludge (at a rate equivalent to 350 kg N ha^{-1}). (a) Leach-1, (b) Leach-2, (c) Leach-3, (d) Leach-4. Incubation was at 22°C . Mean (\pm SE), $n=3$, corrected for mineral N leached from control columns.

The maximum amount of mineral N leached during each *individual* leaching event occurred between day 40 and day 60 post-amendment for all experiments (Figure 3.34). For Leach-1, maximum mineral N was produced on day 57; 7 ± 0 % of applied N as mineral N. The maximum was day 39 for Leach-2, where 6 ± 1 % of applied N as mineral N were produced, with nitrate leached at 4 ± 1 % of applied N as mineral N. The maximum was day 47 for Leach-3, where 7 ± 0 % of applied N as mineral N were leached, with nitrate leached at 5 ± 1 % of applied N as mineral N.

3.6.2 DECOMPOSITION ON A SOIL MATRIX (LEACH-4)

Experiment Leach-4 addressed the question whether similar amounts of mineral N and nitrate N were leached when fellmongery sludge was decomposed on a soil matrix rather than a sand matrix. Traps for volatilised ammonia-N were included in the leaching columns to account for ammonia-N released during fellmongery sludge decomposition. After 35 days decomposition less than 2 % applied TN was recovered as ammonia-N, after which time ammonia trapping was discontinued. More mineral N was leached from fellmongery sludge decomposing on soil (39 ± 1 % of applied N) than on sand (29 ± 0.4 of applied N for Leach-1 and Leach-2 data combined ($n = 6$; $p = 3.48 \times 10^{-6}$). When fellmongery sludge was decomposing on soil, essentially all the mineral N leached was nitrate; 36 ± 2 % of applied N (Table 3.16, Figure 3.34 (d) and Figure 3.35 (d)).

3.6.3 RYEGRASS PRODUCTION FROM FELLMONGERY SLUDGE-AMENDED LEACHING MICROCOSMS

At the termination of the Leach-3 the ten longest ryegrass leaves were harvested from each column. Significantly longer grass blades and significantly more above ground biomass were produced when ryegrass grew in the presence of fellmongery sludge than from ryegrass grown in the non-amended control columns (Table 3.17).

Table 3.17. Leaf length and dry matter production of ryegrass harvested from Leach-3 fellmongery-amended sand columns after 70 days growth. Means (\pm SE), $n = 30$ for leaf length and $n = 3$ for dry matter production.

	Control	Amended	t-test Control vs Amended
Ryegrass Leaf Length (mm)	118 (± 6)	212 (± 4)	$p < 0.001$
Grass Biomass (mg)	507 (± 21)	2870 (± 272)	$p < 0.001$

Leach-4 was used to determine the long-term availability of fellmongery sludge-N to plants. At the termination of Leach-4 ryegrass was sown and the grass allowed to establish for 50 days before harvesting (regular watering but no leachate analysed). The amended soil produced significantly more aboveground biomass, which contained significantly more total plant-N per microcosm than that the controls (Table 3.18). However, there was no significant difference in the TN (% dw) of ryegrass grown on the control soils and that grown on the amended soils.

Table 3.18. Grass harvested from fellmongery-amended columns (Leach-4) after 50 days growth. The total N in grass harvested was the TN of each column added together. Means (SE), $n=3$.

	Control Mean \pm SE	Amended Mean \pm SE	t-test Control vs Amended
Grass Biomass (mg)	105.8 \pm 2.0	165.3 \pm 5	$p < 0.01$
Total N of Grass (% dw)	1.25 \pm 0.02	1.24 \pm 0.03	NS
Total N of Harvested Grass (mg)	1.04 \pm 0.03	1.67 \pm 0.09	$p < 0.01$

NS = Not Significant ($p > 0.05$)

3.6.4 SOIL-PACKED AND RYE-GRASS PLANTED LEACHING MICROCOSMS (LEACH-5)

The soil-packed leaching-microcosms of Leach-4 were re-amended with fellmongery sludge after the ryegrass harvest, thus becoming the columns for Leach-5 (Section 2.7). The aim of this experiment was to determine whether re-amended soil could provide ryegrass with additional N and whether the presence of ryegrass would reduce the amount of N leached. Twenty days after the re-amendment of soil with fellmongery sludge there was lush ryegrass re-growth (Figure 3.36), and less than 2 % of the i-TN had been leached as nitrate (Figure 3.38 (b,d)). The re-amendment of soil with fellmongery sludge had significantly increased the plant biomass and TN after only 20 days of grass growth; 40 % more ryegrass (dry weight), three times more TN (% dw) and four times more nitrate, than that from non-amended soil (Table 3.19).

All the ryegrass in the amended soil died after the ryegrass was harvested on day 20 and no signs of recovery were seen. The ryegrass in control columns re-grew (Figure 3.37), suggesting that the harvesting method was not responsible for the death of grass on the amended soil. After 57 days, with still no ryegrass re-growth in amended soil, it was decided to abandon Leach-5 as the comparisons between control and amended soils were no longer valid: the control soils supported ryegrass and the amended soils did not.

Table 3.19. Average (\pm SE) total-N and nitrate-N of ryegrass harvested from columns of Leach #5 on day 20 post-amendment with fellmongery sludge. The values from t-tests to compare the means are below each column (n = 3).

	Control Mean \pm SE	Amended Mean \pm SE	t-test Control vs Amended
Grass Biomass (mg)	103.0 \pm 2.0	375.1 \pm 7.2	p < 0.001
Total N of Grass (% dw)	1.25 \pm 0.04	3.50 \pm 0.05	p < 0.001
Total N of Harvested Grass (mg)	1.28 \pm 0.03	13.13 \pm 0.44	p < 0.001
Nitrate-N (% of TN)	1.49 ¹	5.91 \pm 0.34	

¹ Insufficient sample was available for replication, therefore the ryegrass from the control columns was pooled for determination of its nitrate content.



Figure 3.36. Leaching microcosms of Leach-5, 20 days post-amendment with fellmongery sludge. Columns on the left were amended with fellmongery sludge columns and those on the right were non-amended controls. Incubation was at 22 °C with a 16-h photoperiod.



Figure 3.37. Leaching microcosms of Leach-5, 60 days post-amendment with fellmongery sludge. Columns on the left were amended columns and those on the right were non-amended controls. Incubation was at 22 °C with a 16-h photoperiod.

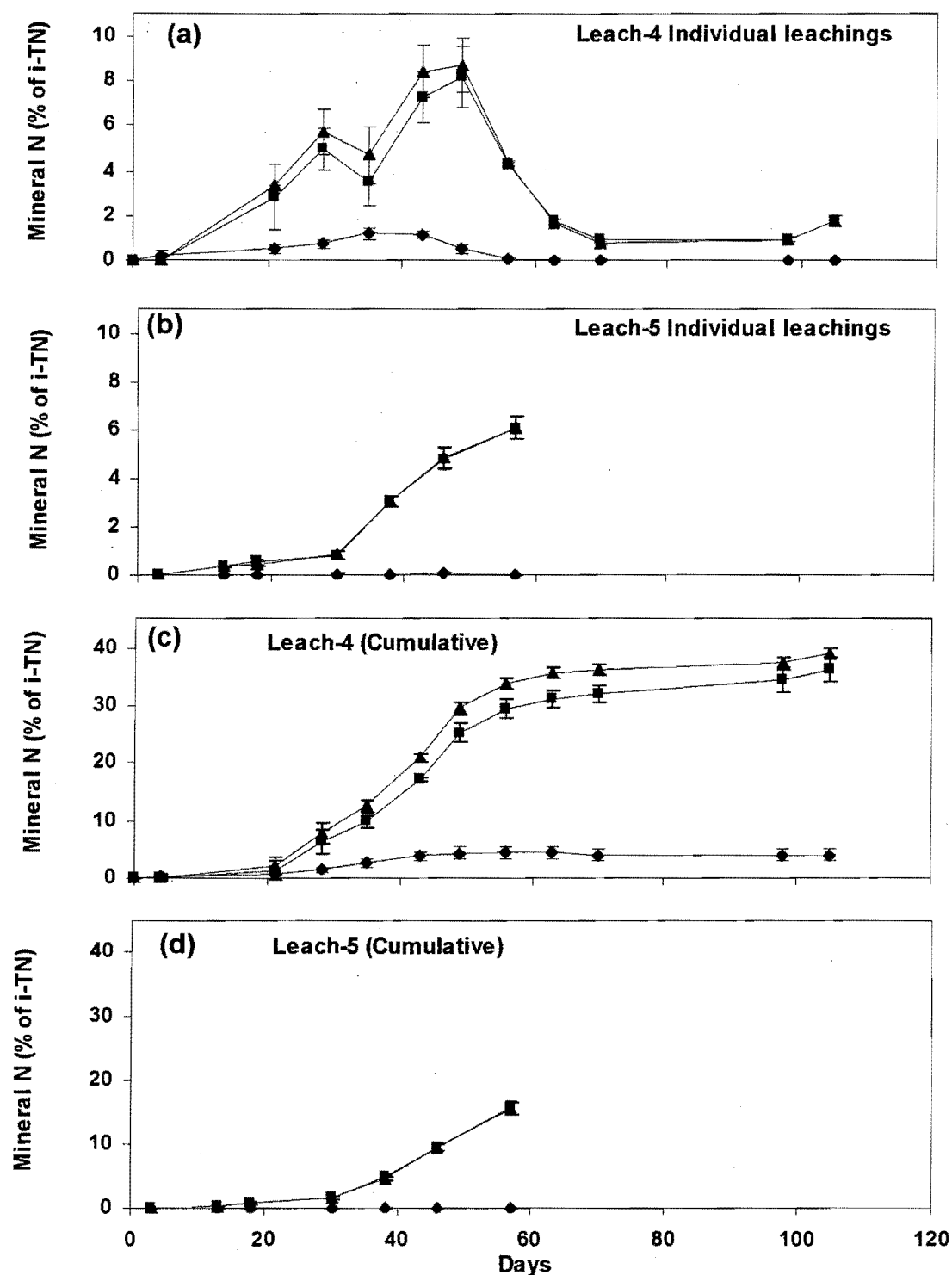


Figure 3.38. Total mineral-N (▲), nitrate-N (■) and ammonium-N (◆) leached from Leach-4 and Leach-5 soil-packed leaching microcosms amended with fellmongery sludge (at a rate equivalent to 350 kg N ha^{-1}). (a) Leach-4 individual leachings, (b) Leach-5 individual leachings, (c) Leach-4 cumulative leaching, and (d) Leach-5 cumulative leaching. Mean (\pm SE), $n=3$, corrected for mineral N leached from control columns.

In Leach-5 the presence of ryegrass had made a large difference to the daily nitrate leached up to 20 days, when the ryegrass in Leach-5 died (Figure 3.38 a, b). With the ryegrass present < 2 % of the applied N was leached as nitrate in Leach-5, which compared to 6 % and 10 % of applied N leached as nitrate after 28 and 35 days, respectively, in Leach-4 (Figure 3.38 c, d). The presence of ryegrass had reduced nitrate leached; cumulative nitrate leached was 29 % of applied TN as nitrate (Leach-4) and 16 % of applied TN leached as nitrate for Leach-5 (Figure 3.38 c, d).

3.7 THE MICROBIAL BIOMASS OF WOOLSCOUR AND FELLMONGERY SLUDGE-AMENDED SOIL

The aim of this set of experiments was to determine whether the amendment of soil with woolscour and fellmongery sludges affected the level of MB, as estimated by the fumigation-extraction technique (H8 in Table 2.5). This question was addressed on the microcosm-scale, which was consistent with the scale of experiments that indicated that woolscour and fellmongery sludges had a negative effect on nutrient cycling (see Figure 3.15 and Figure 3.26). Soil was also amended on a larger scale in a field plot. The MB was determined on amended soil that had been incubated for 10 months at field conditions and also on pots of soil that were incubated for 5 months in the field and 5 months in a glasshouse. This latter experiment addressed the question of whether soil amended with the sludges would respond to the mesophilic incubation conditions of the glasshouse in a manner similar to the non-amended soil (H9 in Table 2.5).

3.7.1 MICROCOSM STUDY

In this experiment, the day that the soil had been amended with fellmongery sludge had an absolute MB of $338 \mu\text{g MB-N g}^{-1}$, which was significantly more than $88 \mu\text{g MB-N g}^{-1}$ for the control soil (Figure 3.39). The increase in MB of fellmongery sludge-amended soil was postulated to be due to freeze-dried organisms being resuscitated during the incubation period that was used after soil preparation, but prior to fumigation.

After 50 days of sludge decomposition, the amended and control soils contained similar amounts of absolute MB-N ($\mu\text{g MB-N g}^{-1}$ soil; Figure 3.39 (a)). The presentation of MB-N data as a percentage of soil TN, which would include N added with the sludge, was used to indicate the amount of TN immobilised in the MB. When the data were graphed in this form a different picture emerged (Figure 3.39 (b)); both amended soils contained significantly more MB-N than the control soil (Table 3.20). This indicated that amendment of soil with woolscour and fellmongery sludges had a significant, and positive, effect on the amount of MB.

After 365 days incubation both amended soils were significantly more acidic than the control soil (Table 3.20). The amended soils had significantly more organic matter than the control soil, however the difference was not considered biologically meaningful (Table 3.20). Both amended soils had significantly more TN after one year than the control soil (Table 3.20), which indicated that amendment had significantly boosted the TN of the soil. The MB of amended soils was below detection level by the fumigation-extraction method after 365 days of sludge decomposition in the microcosms (Table 3.20). A plate count assay indicated that the amended soils did contain culturable organisms, almost exclusively small Gram positive rods without spores, which may suggest that the MB had gone into a survival mode by forming spores, which readily germinated on the culture media (personal communication Dr J. Klena¹⁰). The microcosm method was not considered responsible for the decline in MB-N, as the control microcosms did not have significantly different amounts of MB-N over the incubation period ($p = 0.1285$).

¹⁰ Department of Plant and Microbial Sciences, University of Canterbury.

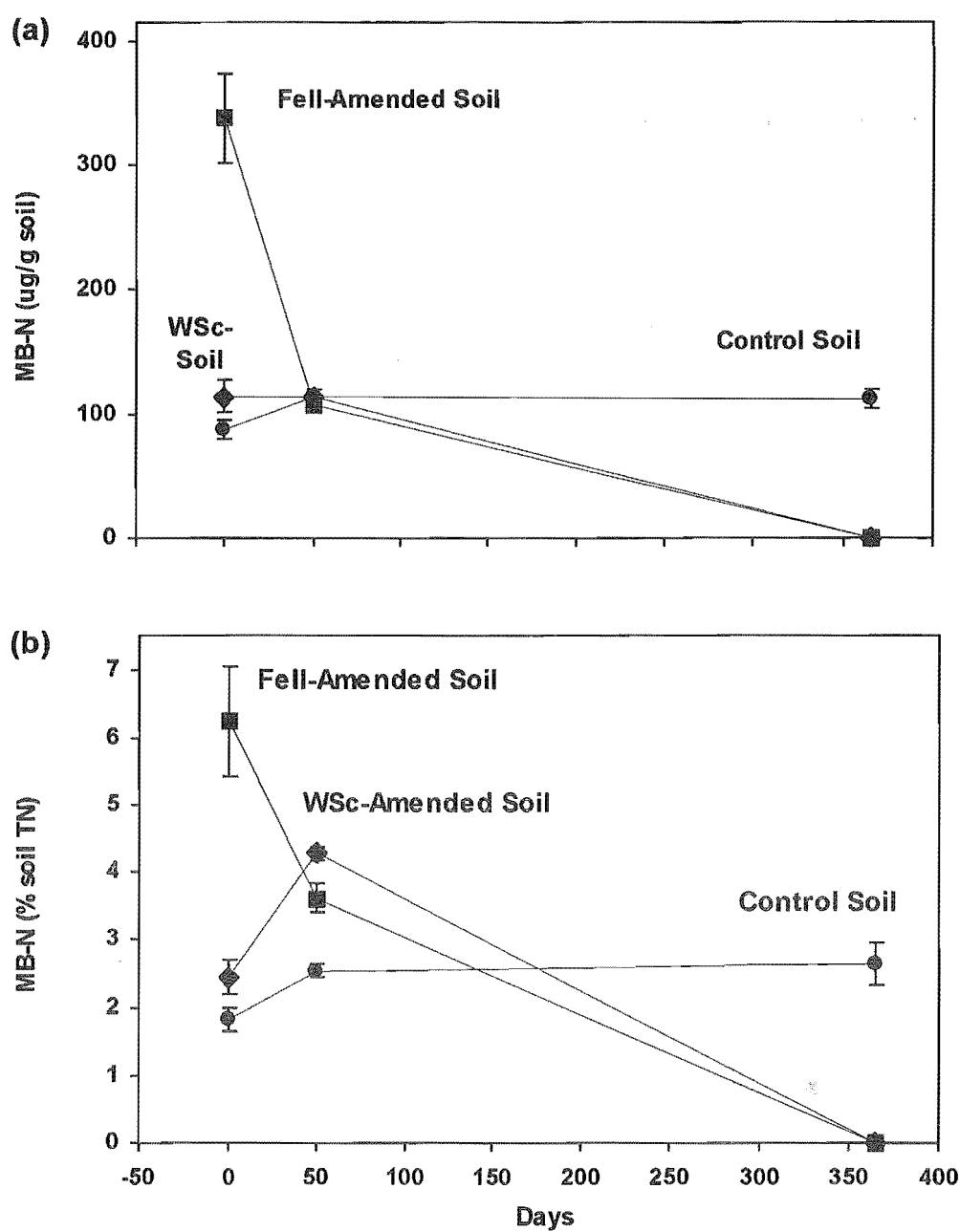


Figure 3.39. The microbial biomass-N of microcosm-incubated control (●) soil and soils amended with woolscour (◆) and fellmongery (■) sludges at 200 kg N ha⁻¹. Units used are (a) $\mu\text{g MB-N g}^{-1}$ soil and (b) MB-N as a percent of soil TN. Incubation was at 22 °C and 20 % moisture. Means (SE), n=6.

Table 3.20. Parameters measured from soils amended with woolsour and fellmongery sludges at 200 kg N ha⁻¹, and incubated in microcosms at 20 % moisture and 22 °C for 365 days. Means \pm SE are presented, n = 6. Means in a row that have the same letter were not significantly different at $\alpha = 0.05$.

	Control Soil		Woolsour-Amended Soil		Fellmongery-Amended Soil	
pH	6.3 \pm 0.02	a	5.8 \pm 0.04	b	5.8 \pm 0.05	b
Organic Matter (%)	11 \pm 0.1	a	12 \pm 0.1	b	11 \pm 0.1	a
Total Nitrogen (%)	0.44 \pm 0.03	a	0.51 \pm 0.00	b	0.52 \pm 0.01	b
MB-N (% of TN)	2.6 \pm 0.3	a	0.0 \pm 0.0	b	0.0 \pm 0.0	b

3.7.2 FIELD STUDY

Field soil, sampled 10 months post-amendment with fellmongery sludge, had a significantly lower pH than other treatments, and the woolsour and fellmongery sludge-amended soils had more TN than the unamended control soil (Table 3.21). Other parameters measured were not significantly different between treatments (Table 3.21; $\alpha = 0.05$). However, trends were similar to those seen in microcosm studies (Section 3.7.1); an increase in the absolute MB-N ($\mu\text{g N g}^{-1}$ soil) and a decrease in soil N immobilised in the MB (MB-N % of TN) for sludge amended soils compared with the unamended control soil (Table 3.21).

3.7.3 GLASSHOUSE STUDY

The surface of amended soils (10 months post-amendment) still showed signs of the applied sludges (Figure 3.40, all plant material had been harvested prior to photograph being taken). This was particularly noticeable for the woolsour sludge-amended soil where a thin crust, presumably resistant woolsour sludge (as judged by the very faint odour), could be seen (Figure 3.40 a).

After 5 months at field conditions and 5 months at glasshouse conditions the MB of the potted soils was estimated using the fumigation-extraction method. The amended soils had more absolute MB-N ($\mu\text{g N g}^{-1}$ soil) than the control soil (Table 3.22). When the MB-N data are presented as a fraction of the soil TN, amended soils showed a trend of decreased MB compared to the non-amended control soil. The differences, however, were not significant ($p > 0.05$).

Table 3.21. Parameters determined on field soil amended with woolsour sludge (woolsour soil) at 60 kg N ha⁻¹ and fellmongery sludge (fellmongery soil) at 195 kg N ha⁻¹ after 10 months at field conditions. Means \pm SE are presented, n = 5. Means in a row that have the same letter were not significantly different at $\alpha = 0.05$.

Field Incubated Soil								
Parameter	Unit	Control Soil		Woolsour Soil		Fellmongery Soil		p
pH		7.0	a	7.1	a	6.7	b	p < 0.001
Organic Matter	(%)	7	a	9	a	8	a	p < 0.01
Moisture	(%)	21	a	23	a	22	a	NS
Total N	(%)	0.29	a	0.38	b	0.34	ab	p < 0.05
Mineral N	(% of TN)	1.2	a	1.2	a	1.4	a	NS
MB-N	($\mu\text{g N g}^{-1}$ soil)	84	a	92	a	86	a	NS
MB-N	(% of TN)	3.0	a	2.4	a	2.5	a	NS

NS = Not Significant

The purpose of incubating field soil under glasshouse conditions was to determine whether the MB of amended soils responded to the consistent climatic environment under glasshouse conditions better than that under field conditions. The MB responded well to the glasshouse conditions, this was regardless of whether the soil had been amended or not (Table 3.23). However, incubation under glasshouse conditions did not affect soil N compared with field-incubated soil. The amended soils had more TN than the control, under field or glasshouse conditions, indicating greater N immobilisation in the MB (Table 3.23).

There was no significant effect on the amount of plant material produced, or the N within the plant material, due to soil treatment (Table 3.24). The plant material from fellmongery sludge-amended soil had 127-mg of plant-TN *c.f.* 108-mg of plant-TN from the control soil (Table 3.24), which was a non-significant trend of increased plant uptake of N on fellmongery sludge-amended soil.

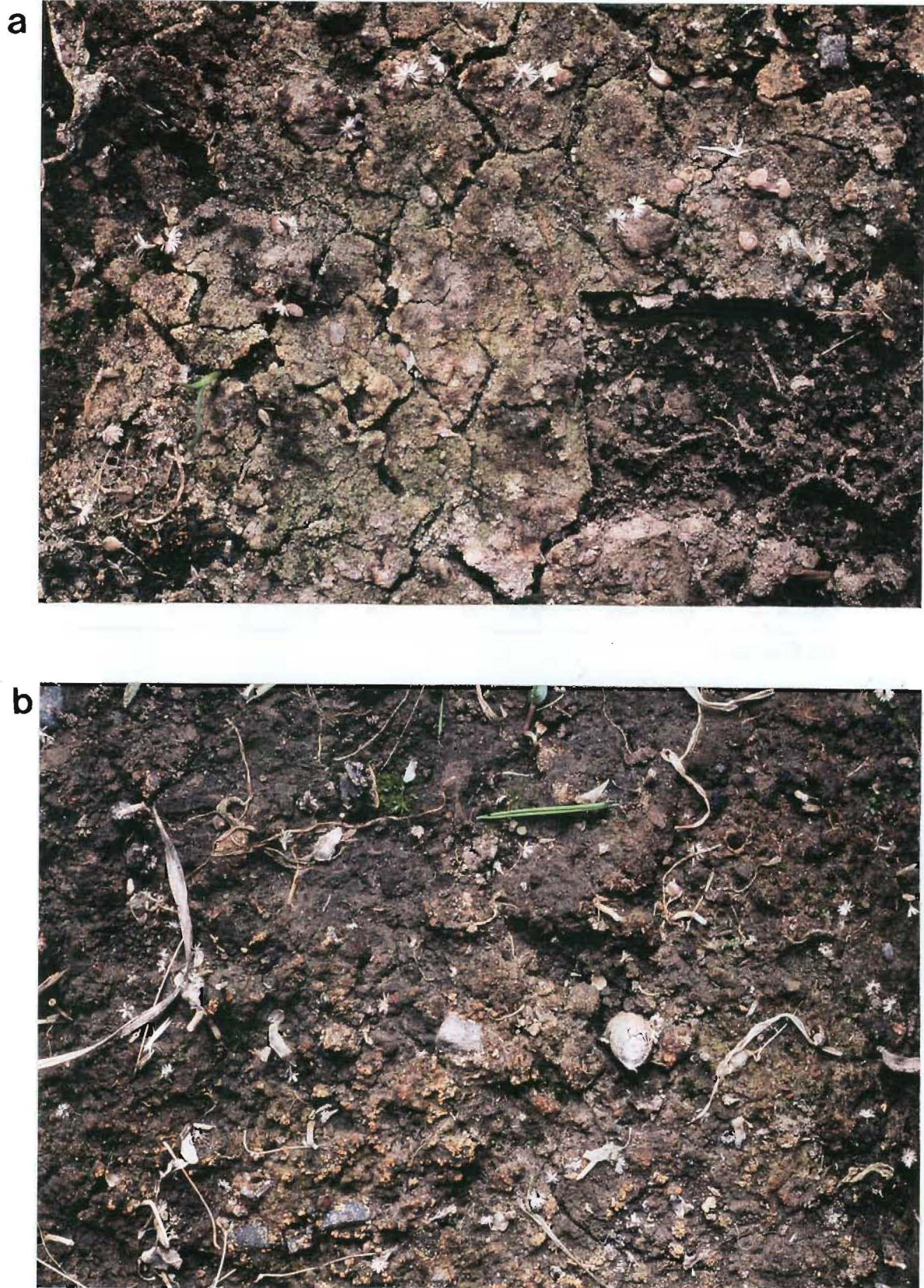


Figure 3.40. The glasshouse-incubated soils amended with (a) woolscour sludge (section cut out of the sludge surface on the right hand side, measured 6 x 6 cm) and (b) fellmongery sludge, 10 months post amendment and after plant harvest.

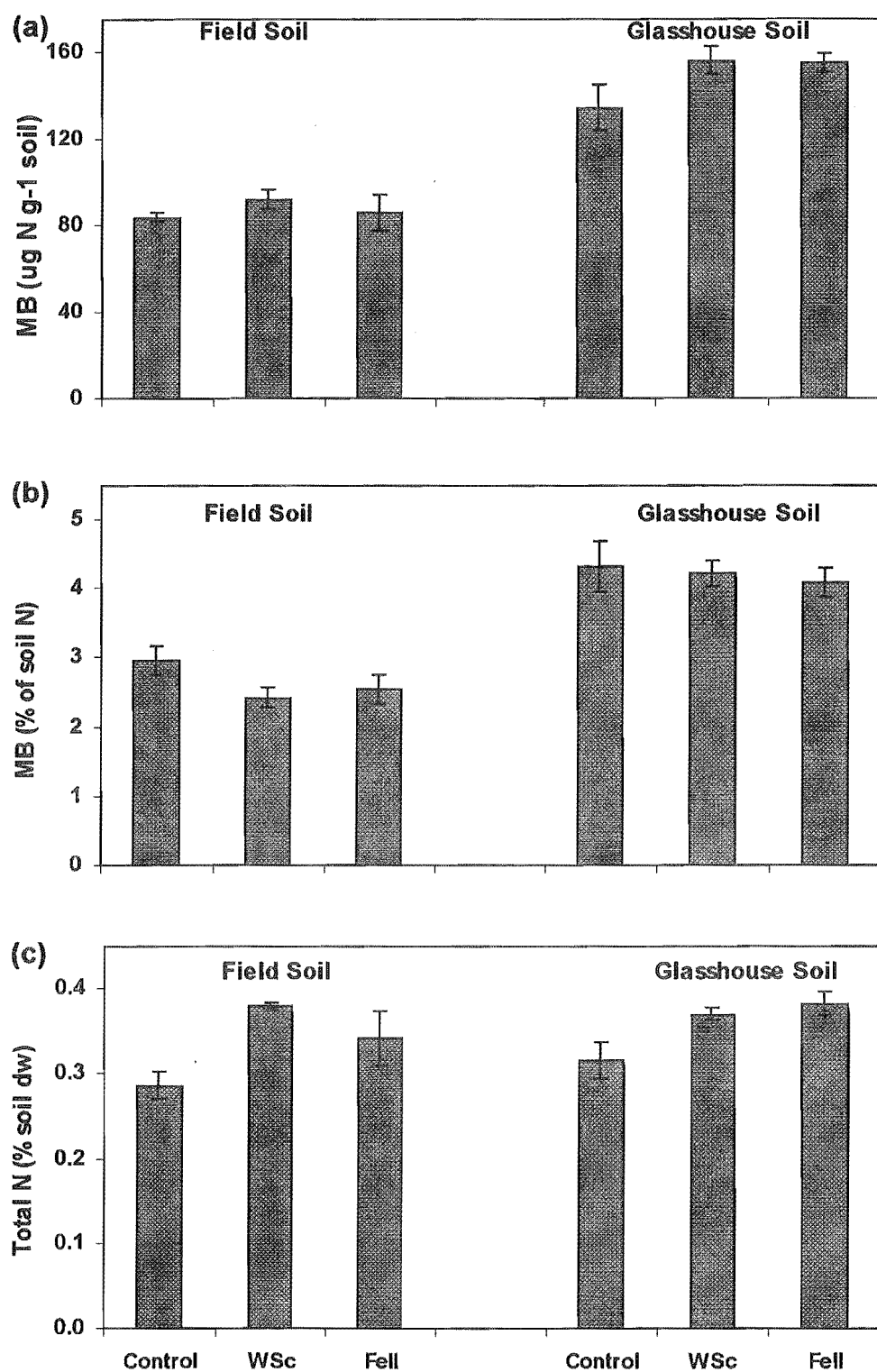


Figure 3.41. Glasshouse-incubated soils amended with woolscour (WSc) at 60 kg N ha^{-1} and fellmongery sludge (Fell) at 195 kg N ha^{-1} (means \pm SE). Soils were amended in the field and incubated at field conditions for five months before being transferred to the glasshouse for a further five months of incubation (at $18^\circ\text{C} \pm 5^\circ\text{C}$).

Table 3.22. Field soil amended with woolscour sludge (woolscour soil) at 60 kg N ha⁻¹ and fellmongery sludge (fellmongery soil) at 195 kg N ha⁻¹ after 5 months at field conditions and 5 months at glasshouse conditions (18 ± 5 °C). Means, n=5. Letters following a mean that are the same per row indicate that the means were not significantly different at $\alpha = 0.05$.

Field-and-Glasshouse Incubated Soil								
Parameter	Unit	Control Soil		Woolscour Soil		Fellmongery Soil		p
Organic Matter	(%)	8.7	a	9.8	b	10.4	b	p < 0.001
Moisture	(%)	23	a	22	a	23	a	NS
Total N	(%)	0.32	a	0.37	b	0.38	b	p < 0.05
MB-N	(mg N g ⁻¹ soil)	134	a	156	a	155	a	NS
MB-N	(% of TN)	4.3	a	4.2	a	4.1	a	NS

NS = Not Significant

Table 3.23. Results from t-tests that compared field-incubated soils vs glasshouse-incubated soils.

	Control vs Control Soil	WSc vs WSc Soil	Fell vs Fell Soil
MB-N (µg N g ⁻¹ soil)	p = 0.0013	p = 3.72x10 ⁻⁵	p < 0.001
MB-N (% of TN)	p = 0.0116	p = 4.65x10 ⁻⁵	p < 0.001
Total Nitrogen (% dw)	p = 0.3141	p = 0.2540	NS

NS = Not Significant

Table 3.24. Plant material harvested from soils amended with woolscour sludge at 60 kg N ha⁻¹ (woolscour soil) and fellmongery sludge at 195 kg N ha⁻¹ (fellmongery soil) after 5 months at field conditions and 5 months at glasshouse conditions (18 ± 5 °C). Means, n=5. Letters following a mean that are the same per row indicate that the means were not significantly different at $\alpha = 0.05$.

Plant Material from Glasshouse Soil Summary						
Parameter	Unit	Control Soil		Woolscour Soil		Fellmongery Soil
Total Plant Biomass	g	8.6	a	8.4	a	11.0 a
TN	% dw	1.31	a	1.12	a	1.17 a
Total Plant Nitrogen	mg	108.1	a	88.9	a	127.0 a
TN in plants	kg N ha ⁻¹	37		31		44



4 DISCUSSION

4.1 THE DECOMPOSITION OF WOOLSCOUR AND FELLMONGERY SLUDGES

The aims of this thesis were to determine the decomposition of a woolscour sludge and a fellmongery sludge, the constraints on sludge decomposition and the consequences of using the sludges to amend soil. The extent to which the sludges decomposed was hypothesised to be similar under ambient conditions (H1 in Table 2.5). This hypothesis was tested using three assessments of decomposition: substrate weight loss, C mineralisation and N mineralisation. Experimental evidence gained using these methods consistently indicated that the fellmongery sludge decomposed more rapidly and to a greater extent than woolscour sludge. Therefore, the initial hypothesis was not supported and the quality of woolscour and fellmongery sludges as substrates for microbial metabolism was considered to be significantly different.

4.1.1 ESTIMATION OF DECOMPOSITION BY WEIGHT LOSS

During 50 days of decomposition at ambient incubation conditions, the fellmongery sludge lost significantly more substrate weight than the woolscour sludge, but significantly less weight than casein (Figure 3.16). Weight loss was an unsuitable method by which to accurately determine the decomposition of woolscour sludge, since the woolgrease appeared to retain moisture, which was not driven off during drying at 105 °C. Assuming that approximately 50 % of the added moisture were retained during the drying of woolscour sludge samples after 50 days of decomposition, a weight loss nearer to 20 % (of initial weight) might be a more accurate description of woolscour sludge decomposition. However, even allowing for the inaccuracy in weight loss data in this case, it seems reasonable to conclude that woolscour sludge lost less weight than fellmongery sludge during decomposition.

Swift *et al.* (1979) outlined a resource quality hypothesis as: “different resource types do consistently decompose at different rates, even under conditions when all other factors, such as those of the physical environment, are controlled”. Based on this description of substrate quality, it is reasonable to conclude that woolscour and

fellmongery sludges were significantly different in the quality of substrate presented to microorganisms for decomposition. Thus, a resource quality sequence of casein > fellmongery sludge >> woolscur sludge was indicated by substrate weight loss during decomposition.

Organic matter loses weight during decomposition by physical, chemical, climatic and biological means (Swift *et al.*, 1979). Abiotic causes of weight loss are processes such as leaching of water-soluble material and weathering by wind and water. Biotic weight loss occurs by enzyme-mediated mineralisation of organic matter, carried out primarily by soil microbes. Microbes rely almost entirely on incoming substrates for energy and nutrients, thus bringing about loss of substrate weight at the site of residue addition (Wardle, 1992). However, other organisms, such as earthworms, seek out organic matter and may remove material from the original resource. This type of weight loss is really "weight transfer", as the material removed still requires mineralisation, and can introduce artefacts when interpreting *in situ* weight loss data. Therefore, microcosm studies are often used gain an understanding of the factors that influence decomposition.

Microcosm studies are highly artificial due to the exclusion of many biotic and abiotic factors. These factors, individually and collectively, determine the rate and extent of decomposition. For example, comminution of a substrate may enhance decomposition by a reduction in particle size and an increase in surface area (Swift *et al.*, 1979). As microcosm studies usually exclude fauna, this type of substrate alteration does not generally occur. However, the use of microcosms when studying decomposition processes, permits unambiguous information about substrate weight loss (and mineralisation) to be gained. The value of using microcosms is that decomposition potential can be determined (Bremner, 1965b).

4.1.2 ESTIMATION OF DECOMPOSITION BY C MINERALISATION

Compared to glucose under ambient conditions, significantly more CO₂ was evolved than from fellmongery sludge. Fellmongery sludge evolved more CO₂ than did woolscur sludge (Figure 3.17). Therefore, as with the weight loss data, the C mineralisation data did not support H1 (Table 2.5) and indicated that fellmongery

sludge presented microorganisms with a substrate that was more readily metabolised than wooldscour sludge.

Carbon mineralisation is a consequence of the aerobic decomposition of organic matter. During decomposition, cellular energy is produced by cleavage of the energy-rich bonds in the organic substrate, and CO₂ eliminated as a waste product (C mineralisation). However, substrates must also provide C units for anabolic processes. The balance for microbes is to have enough energy for cellular maintenance and biosynthesis. The complete oxidation of one molecule of glucose contributes 38 molecules of ATP, making glucose a substrate of high quality (Atlas, 1988). Thus, the percentage of i-TC mineralised can indicate whether a substrate can provide adequate C for both energy production and biomass synthesis. While glucose is generally fully metabolised (Atlas, 1988), other substrates such as leaf litter and sewage sludge will contain a smaller proportion of high quality material, which overall, makes them substrates of lower quality than glucose.

Lower-quality substrates will still evolve CO₂ and produce biomass, but will also have a portion of the substrate-C that persists (Swift *et al.*, 1979; Terry *et al.*, 1979; Parker and Sommers, 1983). The total time for complete mineralisation of organic material depends on substrate quality and environmental conditions (physical and biological). Plant-derived C may persist for up to 2,000 years (Swift *et al.*, 1979), while sewage sludge C is predicted to take 300 to 900 years for complete mineralisation (Terry *et al.*, 1979). This suggests that large portions of the organic matter are recalcitrant over the usual time that decomposition studies are done.

Difficulties may arise in the interpretation of evolved CO₂ data. An energy-rich substrate such as glucose tends to evenly divide its C between respiration and MB synthesis, as seen in Figure 3.17 where 50 % of added glucose-C was respired. However, under stressful conditions, respiration may be high, indicating metabolically active organisms, but no accumulation of MB occurs (Killham, 1985). The ratio of respired-C-to-MB-C tends to increase with increasing stress (Killham, 1985). For example, microbes in saline environments need to expend more of their energy in retention and exclusion of intracellular ions against concentration gradients and to synthesise internal osmotic agents (Killham and Firestone, 1984). Similarly, metal-contaminated soil tended to show a reduction in MB compared to soil low in metals, whereas the cellular energy (as measured by ATP) was equivalent between the two soils

(Chander and Brookes, 1992). The amendment of metal-contaminated soil with ^{14}C -glucose indicated that heavily contaminated soil respired significantly more $^{14}\text{CO}_2$ than control soil, while at the same time the ^{14}C -MB in the contaminated soil was significantly lower than the control (Bardgett and Saggar, 1994). There was also a significant increase in both the respired-C:MB-C ratio and the respired- ^{14}C :MB- ^{14}C ratio of soil heavily contaminated with metals. This indicated that the presence of heavy metals had reduced the MB and the organisms that survived respired more C than they were immobilising in new biomass.

Sewage sludge may be an appropriate substrate with which to compare the decomposition of woollscour and fellmongery sludges, since it is also derived from animal-based products, although sewage sludge has undergone extensive microbial modification (Section 1.5.1). The amount of CO_2 produced during the decomposition of fellmongery sludge (42 % i-TC in 82 days, Figure 3.17) was similar to the higher end of a range reported from decomposing sewage sludge (26 to 42 % of i-TC in 130 days), when incubated at ambient temperatures (Terry *et al.*, 1979). This suggested a similarity in substrate quality between sewage and fellmongery sludges. A possible order of substrate quality based on C mineralisation might be, glucose > fellmongery sludge > sewage sludge > woollscour sludge.

The effect of soil stress on microorganisms is particularly relevant when studying wastes such as sewage sludge, which tend have elevated levels of metals (Table 3.12; (Terry *et al.*, 1979; Fliebbach *et al.*, 1994)). Sewage sludge application to soil boosted respiration and MB-C compared with an untreated soil (Fliebbach *et al.*, 1994). At low sewage sludge application rates, soils tended to have lower respired-C:MB-C ratios than controls, indicating an overall beneficial microbial response to the application of sewage sludge (Fliebbach *et al.*, 1994). However, when the rate of sewage sludge application was increased, so too was the respired-C:MB-C ratio compared to the control soil. Soil that had consistently received sewage sludges low in metals responded better than soils receiving high-metal sludges (Fliebbach *et al.*, 1994).

Therefore, CO_2 data may suggest no adverse effects on the MB due to sludge addition, when in fact, negative effects are occurring. In these cases the respiration rate may be high due to cellular energy demands, yet the growth of the organisms may be reduced compared to non-stressed environments. Thus, high respiration is not,

necessarily, promoting nutrient cycling and may not be indicative of a resource of high quality.

With no data available with which to compare the CO₂ respired during the decomposition of the woolsour and fellmongery sludges, it is not clear whether elevated levels of respired CO₂ are being observed. Based on weight loss and C mineralisation data, the fellmongery sludge had 36 to 41 % of the substrate decomposed. As weight loss and CO₂ evolution measure the release of similar components, these data can be considered to reflect the extent of fellmongery sludge decomposition, over a period of about 50 days. On the other hand, the weight lost during the decomposition of woolsour sludge was 10 to 20 %, while based on CO₂ evolution 30 % of the sludge was decomposed. The wider range of decomposition data for woolsour sludge may indicate elevated respiration consistent with microbial stress.

4.1.3 ESTIMATION OF DECOMPOSITION BY N MINERALISATION

Under ambient conditions, decomposing casein mineralised 68 % i-TN (Figure 3.27), the fellmongery sludge mineralised 55 % i-TN and the woolsour sludge mineralised 9 % i-TN (Figure 3.19). These N mineralisation data were consistent with weight loss and C mineralisation data and indicated that woolsour sludge decomposed to a lesser extent than fellmongery sludge (Figure 3.19). Returning to a sewage sludge example, Terry *et al.* (1981) reported that 27 to 37 % i-TN was mineralised under ambient conditions. Therefore, based on N mineralisation, and consistent with CO₂ evolution, the substrate quality of sewage sludge appeared to fall between fellmongery and woolsour sludge (Terry *et al.*, 1981).

The mineralisation of sewage sludge-N was reasonably predicted ($r = 0.77$) by the organic N content of the sludge using Equation 4.1 below, which is taken directly from Parker and Sommers (1983). Using Equation 4.1, it was predicted that 33 % and 66 % i-TN would be mineralised during the decomposition of woolsour and fellmongery sludges, respectively. For both sludges this was higher than the N mineralised experimentally, which would suggest that sewage sludge had slightly more labile organic N than fellmongery sludge (and considerably more labile N than woolsour sludge). However, fellmongery sludge-N mineralisation was reasonably predicted using Equation 4.1, whereas woolsour sludge N mineralisation was not. This

suggested that the substrate quality of woolscour sludge-organic N was significantly lower than that of fellmongery and sewage sludge. Thus, based on N mineralisation, the substrate quality sequence indicated was casein > fellmongery sludge > sewage sludge >> woolscour sludge.

$$\% \text{ of added sludge N mineralised} = 6.37 \times \% \text{ sludge organic N} + 4.63 \quad \dots (\text{Equ. 4.1})$$

Based on weight loss and CO₂ evolution data, and using the estimate from Equation 4.1, it was reasonable to expect woolscour sludge to show N mineralisation between 20 and 30 % i-TN. There are three probable reasons why woolscour sludge-N was not extensively mineralised. Firstly, the sludge N was relatively unavailable to microbes, which would be consistent with a substrate of low quality. Secondly, extensive N immobilisation occurred during decomposition, if this were the case, a large increase in the MB would be predicted (see Section 4.5 for discussion of the MB of soil amended with woolscour sludge). Thirdly, that the sludge contained compounds toxic to N mineralisation, with heavy metals and pesticide residues prime candidates.

The analysis of woolscour sludge for heavy metals indicated elevated levels of Zn and Cd (Table 3.12), unfortunately pesticide analysis was not performed due to financial constraints. It is assumed, however, that as many of the pesticides associated with wool production are lipophilic (Hassall, 1990; Aislabie and Lloyd-Jones, 1995; Greer, 1997), if pesticides were present, then the woolscour sludge (33 % grease) would have a greater concentration than the fellmongery sludge (6 % grease; Table 3.1).

In 1987 an International Wool Secretariat survey identified pesticides and their residues as the primary concern to wool producers, due to their presence in refined woolgrease products and in woolscouring wastes (Shaw, 1990). It is estimated that approximately 60 % of the pesticides on the annual wool harvest are present in resulting woolscouring wastes and most of the remainder in the recoverable woolgrease, the material from which lanolin is refined (Russell, 1996). Pesticide levels were found to be unacceptably high in pharmaceutical preparations based upon lanolin, especially baby care products, where pesticide levels cannot exceed 1-3 ppm (Johnson, 1990). Therefore, since the international wool community has expressed concern about pesticides in woolgrease and woolscour wastes, it is highly probable that the woolscour

sludge (and to a lesser extent the fellmongery sludge) used during this research would have contained pesticides.

The pesticides applied to sheep are usually intended to control insect parasites. In general the pesticides work by inhibiting enzymatic function, thus disrupting processes such as the regeneration of acetylcholine in synaptic junctions, and inhibiting the activity of esterases and synthesis of chitin (Hassall, 1990; Greer, 1997). However, insecticides can also inhibit enzymatic processes in non-target organisms, with fungi tending to be more susceptible than bacteria (Colinas *et al.*, 1994). If any group of soil organisms were specifically reduced by the presence of pesticides, then inhibition of soil activities in general, such as N mineralisation, may result. In addition, microbial metabolism depends on extracellular enzymatic function, and if these enzymes were inhibited then the quantity of substrate available for cellular maintenance and biosynthesis would be drastically reduced and the decomposition of organic matter would also be reduced (Sparling, 1997).

An alternative explanation for the low level of N mineralisation seen during the decomposition of woollscour sludge might be the level of metals associated with the sludge. An analysis of the metals in woollscour sludge indicated that Zn was present at the maximum recommended level and Cd was present at more than twice the maximum recommended level (Table 3.12).

Heavy metals, including some trace metals, are metals with an atomic density greater than 6-g cm^{-3} (e.g. Cd, Cr, Cu, Hg, Ni, Pb and Zn). The term "heavy metal" is often used to describe material contaminated with non-essential metals (e.g. As, Cd and Hg) or excessive amounts of trace metals such that they are causing toxicity (Alloway and Ayres, 1997). Heavy metals inhibit many enzymes, leading to the disruption of cellular function (sometimes causing cell death), with the flow-on effect that organisms fail to carry out essential processes. In the soil environment this may lead to a reduction in the decomposition of organic matter and inhibit processes such as the conversion of nitrite to nitrate, causing an accumulation of nitrite (Chaney *et al.*, 1978; Liang and Tabatabai, 1978; Chang and Broadbent, 1982; Dahlin *et al.*, 1997).

Heavy metals usually inhibit enzymes by occupying or disrupting the substrate attachment site(s), which can affect, amongst other things, membrane integrity, prevent redox reactions and interfere with phosphorylation (Stryer, 1981). Most organisms, and microbes in particular (usually *via* resistance plasmids (Brock *et al.*, 1984)), can tolerate

some fluctuation in heavy metals, which permits normal functioning to continue before enzymatic function is compromised (Alloway and Ayres, 1997). Zinc and Cd are two heavy metals that organisms tend to tolerate by the formation of metallothionein proteins, which attach the metals to -SH groups and the metal-protein complex is then excreted (Alloway and Ayres, 1997). It is therefore possible that microbes associated with the woolscour sludge may not have been as susceptible to the particular metals that were elevated in the sludge (Zn and Cd).

There is a natural background level of metals in the environment due to weathering of rocks and minerals. Hence it is not the absolute amount of a metal in soil that is indicative of pollution or enrichment, but rather the relative amount of the metal compared to other soils of similar geological form, age and weathering. Many of the metals present in the environment are essential for the normal function of enzymes and are required in small quantities; such metals are referred to as trace metals (Alloway and Ayres, 1997). Most trace metals function as an integral, often covalently bound, part of enzymes, where they alter the active site to facilitate substrate binding (Stryer, 1981).

In the field, sheep collect significant quantities of soil on their wool, and the purpose of the initial washing steps in fellmongering (Figure 1.2) and the entire woolscouring process is to remove this, and other contaminants (Stewart, 1983). It would be expected, therefore, that woolscour sludge (and to a lesser extent the fellmongery sludge) would tend to contain a large quantity of soil. The woolscour sludge, used during this research, contained only 5 % less SiO₂ than a sample of Ilam soil, whereas the fellmongery sludge contained 35 % less SiO₂ (Table 3.10). This indicated that the woolscour sludge did indeed contain a large amount of soil. If the soil that adhered to the fleece were enriched with heavy metals then the woolscour sludge would be similarly enriched, and thus, the extent of sludge decomposition may be reduced.

The presence of Zn and Cd in woolscour sludge can be reasonably accounted for on the basis natural accumulation and of fleece contamination "*in situ*". New Zealand soils, which are naturally sufficient in Zn (McLaren and Cameron, 1990), tend to become contaminated with Cd where they have received applications of phosphate fertilisers, due to Cd in the source rock (Loganathan *et al.*, 1995). In addition, Zn has been established as an essential element for healthy skin development and wool growth in sheep (Masters *et al.*, 1985), and would be expected to be present in skin and wool

fragments. The enrichment of woolscour sludge with soil- and fleece-borne Zn would be further enhanced due to strong sorption of Zn to organic matter (e.g. woolwax) and clay (Viets and Boawn, 1965), both of which are the fleece contaminants that woolscouring aims to remove.

There is however, a further complicating factor that may contribute to the amount of soil associated with woolscour sludge. Elling and Zahan (1989) found that even after careful removal of contaminants from *scoured* wool, the fibres still contained a large amount of ash, suggesting that mineral soil was within the actual wool fibres. Entrained soil, which appeared to enter the wool through frayed tips (Elling and Zahan, 1989), may account for elevated levels of metals, such as Cd, Fe and Zn, associated with the tips of wool when compared with wool taken from near the sheep's skin (Wojciechowska *et al.*, 1992). Wool fibres, especially near the tips, that have been damaged or weakened during growth, tend to break during scouring and contribute to the fibre portion of the woolscour wastes (Stewart, 1983).

As was the case with putative pesticides in woolscour sludge, heavy metals also inhibit decomposition by compromising the enzymatic function of microbes. The affinity of most metals to organic matter means that they adsorb (electrostatically), which tends to make them less bioavailable, however metals are prone to become mobile depending on the pH of the microenvironment (McLaren and Cameron, 1990; Alloway and Ayres, 1997).

4.2 CONSTRAINTS ON THE DECOMPOSITION OF WOOLSCOUR AND FELLMONGERY SLUDGES

The evidence gained during the decomposition experiments discussed above did not support the initial hypothesis; that the extent to which the two sludges decompose was similar under ambient conditions. Therefore, this next section of the research aimed to understand the constraints on N mineralisation during woolscour and fellmongery sludge decomposition (H2 and H3 in Table 2.5). This was achieved by altering the incubation conditions (temperature, moisture, atmosphere, matrix and co-incubation of substrates) and measuring the N mineralised during sludge decomposition. In general, it was found that the extent to which fellmongery sludge-N was mineralised

was strongly dependent on the prevailing incubation conditions. On the other hand, the mineralisation of N during the decomposition of wooldscour sludge was not particularly sensitive to incubation conditions, except temperature.

Nitrogen mineralisation is a reliable technique to determine the extent of organic N transformation into the mineral components of ammonia, ammonium, nitrate and nitrite. The more readily a substrate mineralises its organic N, the higher the quality of material available for microbial metabolism. The principal driving force of microbial metabolism is the production of ATP. Part of the quality of a substrate is the potential to lose electrons (oxidation) and in doing so release sufficient free energy for ATP formation. When oxygen is the terminal electron acceptor, maximum free energy is released as the oxidation process can take organic substrates through to their mineralised products, e.g. CO_2 and NH_3 . Thus aerobic N mineralisation studies indicate a maximum potential of substrate quality. The limitation of these studies is that they are generally, as in the work presented here, performed in microcosms. This limitation is accepted as necessary to obtain unambiguous results that can be interpreted as showing the potential of a substrate to mineralised N.

There are difficulties in reliance on N mineralisation data to judge decomposition, since N immobilisation occurs concomitant with N mineralisation. Net-N mineralisation indicates N released from organic matter in excess to the N requirements of the MB. There are two main advantages to using net-N mineralisation as a parameter by which to quantify decomposition. Firstly, net-N mineralisation represents N that completes the nutrient cycle, i.e. the N available for plant uptake and therefore primary production. Secondly, for N to be mineralised proteins, polypeptides and amino and nucleic acids must be released from complex structures, suggesting that a loss of substrate integrity occurs.

At any point where a decrease in net-N mineralised was recorded, denitrification is a possible explanation. Denitrified-N was not measured during these experiments, and the assumption was made that denitrification would be minimal since the microcosms were aerobically maintained at approximately 20 % moisture. However, even in aerobic soils, microsites exist that have sufficiently low redox potential to facilitate denitrification, thus the potential for some mineral N to be lost *via* this mechanism exists. Fellmongery sludge did not appear to be prone to denitrification; similar amounts of mineral N were leached under two leaching regimes that added

significantly different amounts of water (Leach-1 and Leach-2 in Table 2.8, these data are discussed fully in Section 4.4 below). Hence, although denitrification was not used as an explanation for the results that follow, it was none-the-less a possibility.

4.2.1 THE EFFECT OF TEMPERATURE ON N MINERALISATION

Fellmongery sludge mineralised almost twice as much N at 22 °C than at 10 °C (Figure 3.20). However, there was no net-N mineralisation when fellmongery sludge was decomposed at 43 or 50 °C. Mesophilic microorganisms have an optimum temperature of approximately 25 °C. Either side of 25 °C is a narrow range of temperatures, at which microbial metabolism is almost optimal. The width of this temperature range tends to vary between mesophilic organisms more than the optimum temperature (Atlas, 1988). Exposure to temperatures outside the optimal range significantly alters the metabolic activities of microorganisms. For example, there is an approximate doubling of enzymatic activity with each 10 °C increase in temperature from 0 to 30 °C (Killham, 1994). Therefore, N mineralisation during the decomposition of fellmongery sludge was consistent with mesophilic enzyme activity (Swift *et al.*, 1979; Killham, 1994), and suggested that fellmongery sludge was decomposed by a principally mesophilic microbial population.

Woolscour sludge also showed N mineralisation consistent with decomposition by mesophilic microorganisms. However, unlike fellmongery sludge, woolscour sludge continued to mineralise N when the incubation temperature was increased to 43 and 50 °C (Figure 3.20). At 50 °C, 40 % i-TN was mineralised, which was the highest level of decomposition seen for woolscour sludge at any time during this research (Figure 3.20). Therefore, the gradual increase in net-N mineralisation with increasing temperature is consistent with woolscour sludge being decomposed by two pools of microorganisms, one mesophilic and one thermophilic. The consistent and gradual nature of the temperature profile suggests that the thermophiles are facultative in their metabolism.

Some of the woolscour sludge-N mineralised at 50 °C may be due to the re-mineralisation of N previously immobilised in the cells of mesophiles or denaturation of the wool fibres, making the N more bioavailable. In addition, at the higher temperature there may have been less competition for N by reducing the activity of mesophiles. Thus, mineral N accumulated in the sand matrix. The accumulation of mineral N also

suggested that the thermophilic microbial population was adapted to high C-to-N ratio materials, such that they were not strongly dependent on readily mineralised N. Such a group of microbes would tend to utilise substrates for the C (energy) more than for the N. This suggested that, even though the woolscour sludge had a low C-to-N ratio (8.3), the apparent unavailability of N implied a substrate with an effective C-to-N ratio well above that determined by chemical analysis.

The improved N mineralisation seen when woolscour sludge was decomposed at 50 °C suggested that the presence of grease and heavy metals (and assumed pesticide residues) were not inhibitory to the enzymes expressed by the thermophilic microbial complement. Thus, assuming a consortium of facultative thermophilic microbes decomposed woolscour sludge (which is reasonable given the consistent increase in mineralisation as temperature increased), Zn and Cd at 308 and 7 ppm, respectively, had not irreversibly inhibited N mineralisation (Section 4.1.3).

There is evidence that facultative thermophilic microbes are associated with the dramatic increase in temperature of tightly packed wool bales that contained damp raw wool (Dye, 1964a; Dye, 1964b; Rothbaum and Dye, 1964). The self-heating of wool was a problem for the wool industry at the turn of the century, and in 1906 four of the six ships that left New Zealand loaded with wool bales caught fire when bales of raw wool spontaneously ignited (Walker, 1998). This problem, which ceased to trouble wool exported from Australia by 1900, continued to cause substantial loss to the New Zealand wool industry up to about 1963. In 1963 the spontaneous combustion of wool bales was identified as being strongly microbially mediated, but was dependent on the inclusion of pie wool in a compressed wool bale. Pie wool was (but is not now) the wool recovered after the partially rotting of skin trimmings produced during fellmongering (see Figure 1.2).

Until 1963, pie wool was directly baled in its wet state, with sub-cutaneous fat from the rotted flesh contaminating much of the wool. The evolution of CO₂ and isolation of microbes from the inner part of such wool bales were strongly positively correlated with the increase in temperature, up to 70-80 °C. Thus, microbial activity was considered responsible for the increase in heat, but only under moist conditions; there was little microbial growth and minimal CO₂ evolved from scoured wool, which was dried before baling (Dye, 1964a; Dye, 1964b; Rothbaum and Dye, 1964). Therefore, the material associated with raw wool, including wet pie wool, was sufficient

to sustain considerable microbial growth, though there was minimal damage to the wool fibres (unless they ignited) (Dye, 1964a; Rothbaum and Dye, 1964). The problem of bales of wool igniting has been overcome by exporting mainly scoured wool.

Thus, the enhanced decomposition of woolscour sludge at elevated temperatures may indicate the potential for the sludge to respond well to composting. However, the sludge may not respond well to land application, which does not provide the elevated temperatures that are, seemingly, required for extensive decomposition of the sludge.

4.2.2 THE EFFECT OF MOISTURE ON N MINERALISATION

The decomposition of woolscour sludge was not affected by low (5 %) or moderate (35 %) moisture levels (Figure 3.5), with aerobic conditions still considered to be present at 35 % moisture (Stanford and Epstein, 1974). Nitrogen mineralisation ranged from 3 to 5 % (i-TN) over these moisture levels, which were low even for woolscour sludge and suggested temporal subsample variation (see Section 3.4).

The amount of fellmongery sludge-N mineralised at low moisture was reduced compared to higher moisture levels (Figure 3.21) and it is possible that toxic metabolites (which would have had restricted diffusion away from the substrate) may have inhibited further decomposition at 5 % moisture (Harris, 1988a). Fellmongery sludge did not appear prone to denitrification at the moisture levels used during decomposition experiments, judged by the similarities of N mineralised at moisture ranging from 10 to 35 % (Figure 3.21). While the microcosms were considered aerobic at 35 %, anaerobic microsites may have developed during rapid decomposition of fellmongery sludge (Killham, 1994). Therefore, if such sites did develop, they did so irrespective of the level of moisture. Alternatively, if anaerobic microsites had occurred, anaerobic conditions did not favour decomposition of fellmongery sludge thus accounting for the similarity in mineralisation. Therefore, woolscour and fellmongery sludges have the potential to decompose over a broad range of aerobic soil moisture levels, with the order of substrate quality remaining as fellmongery sludge >> woolscour sludge.

Water is an essential solvent and required by all living systems. As solute concentration (osmotic potential) increases the available water decreases and the soil matrix attracts water more strongly, thus it becomes more difficult for the remaining water to be removed (McLaren and Cameron, 1990). This is due to cohesion (water to

water forces), adhesion (water to mineral surface forces) and capillary action (within the pores of soil particles). These factors represent the amount of force that must be applied (by plants and microbes) to remove water for biochemical processes (McLaren and Cameron, 1990). Water activity (A_w) is an index of water that is free for chemical reaction (free/available water) (Atlas, 1988). At a A_w of 1.0 (equivalent to air of relative humidity at 100 %), microbial growth is usually optimal but falls rapidly as A_w decreases. Fungi tend to tolerate a far lower A_w than bacteria, although most microbes enter survival modes in response to drying (Atlas, 1988). Optimum moisture levels for microbial activity, and N mineralisation specifically, in soil are usually between 0.15 (moist) and 0.5 (drier than 0.15 bar) bar (Stanford and Epstein, 1974; Swift *et al.*, 1979; Terry *et al.*, 1981), or approximately -10 kPa (McLaren and Cameron, 1990).

The moisture level of the medium in which decomposition is occurring needs to be sufficient to facilitate the movement of solutes (food and wastes) to and from decomposer organisms, but low enough to ensure adequate oxygen supply for aerobic metabolism (Payne and Gregory, 1988). Therefore, a reduction in metabolic activity may occur due to low available water in dry soil or where the moisture level itself is not particularly low but the concentration of solutes is high (Harris, 1988a).

4.2.3 THE EFFECT OF ANAEROBIC CONDITIONS ON N MINERALISATION

The woolscour sludge mineralised 5 % (i-TN) and the fellmongery sludge mineralised 15 % (i-TN) after 108 days under anaerobic conditions. Based on the decomposition patterns for woolscour and fellmongery sludges (Figure 3.19 and Figure 3.23 (b)) this indicated that the decomposition of woolscour sludge was not greatly affected by anaerobic conditions, but fellmongery sludge was. This supported the suggestion that the decomposition of woolscour sludge was carried out, primarily, by a different portion of the microbial population to that decomposing fellmongery sludge.

It is possible that fellmongery sludge (more so than woolscour sludge) was C (energy) limited during decomposition, based on the ratio of evolved CO_2 -C-to-N mineralised (Figure 4.42 below). If this were the case, a lower amount of net-N mineralisation would be expected; this occurred during the decomposition of fellmongery sludge (Figure 3.22). Fellmongery sludge had a ratio of 0.7, which means that for every seven units of C mineralised, ten units of N were mineralised, suggesting

that the MB required more C to permit efficient immobilisation of N. In comparison, woolscour sludge had an evolved CO_2 -C-to-N mineralised ratio of about two (after the initial lag period for N mineralisation), which suggested that available N may have limited utilisation of substrate C (Figure 4.42 below).

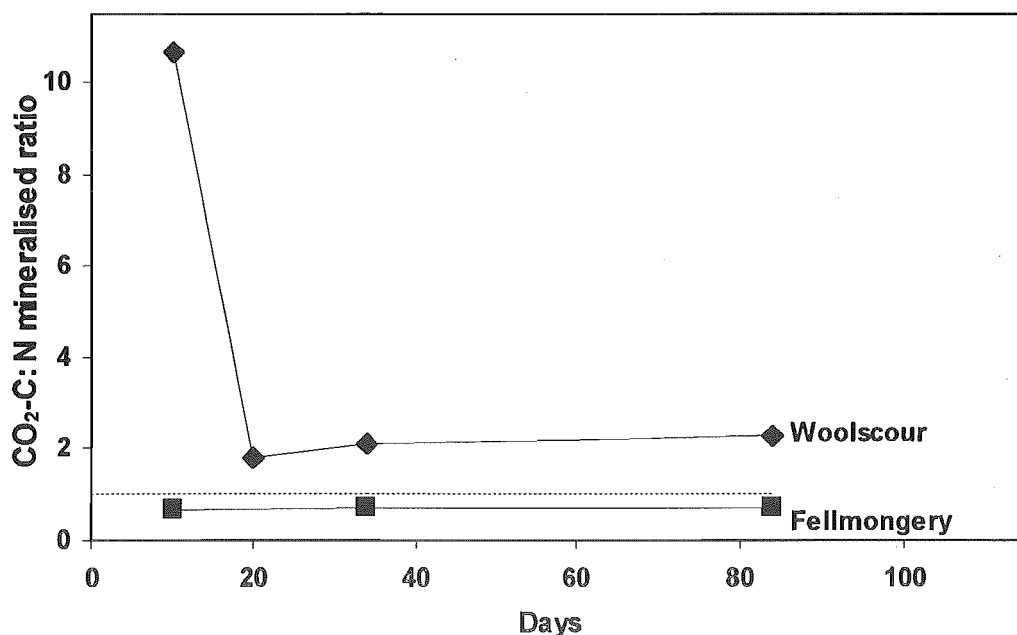


Figure 4.42. Ratio of evolved CO_2 -C-to-N mineralised for woolscour (◆) and fellmongery (■) sludges in sand-based microcosms at 22 °C and 20 % moisture. The dotted line (····) represents a ratio of 1, where C and N mineralisation are equal.

The microbial population associated with the degradation of fellmongery sludge may have been marginally energy limited under anaerobic conditions, which meant more of the sludge would tend to persist in the environment. If this were the case, it is suggested that landfill would not be a reasonable disposal option for fellmongery sludge. If the decomposition of the woolscour and fellmongery sludges were energy limited, then N mineralisation would be reduced irrespective of the microbial complement present. Further, the anaerobic N mineralisation data suggested that fellmongery sludge was decomposed mainly by aerobic organisms. One of the consequences of the aerobic decomposition of a substrate that readily mineralises N is the potential to leach nitrate. This suggested that fellmongery sludge, more than woolscour sludge, required a determination of its mineral N leaching, since the fellmongery sludge is currently disposed of on agricultural land (see Section 4.4 below).

Sewage sludge, like fellmongery sludge, is often applied to land. However, before sewage waste is considered suitable for land-based disposal it is, usually, stabilised. The stabilisation of sewage sludge is usually achieved by anaerobic microbiological treatment, which reduces the N content through denitrification. The stabilised sludge had a lower BOD than non-treated sludge (Alloway and Ayres, 1997). The reason why the anaerobic treatment (of sewage sludges) produces a substrate of lower substrate quality is that anaerobic respiration produces less ATP than aerobic respiration. This is due to the difference in the relative energies between the substrate and the electron acceptor; the higher the redox potential of the electron acceptor, the more ATP can be generated (Harris, 1988b). When molecular oxygen is the electron acceptor the redox potential is +820 mV, when NO_3^- is the electron acceptor during denitrification the redox potential is +420 mV, and in fermentation (where organic matter accepts electrons) the redox potential is +400 mV (Harris, 1988b; Killham, 1994). Therefore, anaerobic metabolism is not as efficient at energy production as aerobic respiration. During anaerobic decomposition more of the material that is readily degradable is utilised by the anaerobic microorganisms, making the digested sludge a substrate of lower quality than the raw sludge (Epstein *et al.*, 1978).

Sewage sludges are extremely variable in their chemical composition and biological properties due to the materials entering the sewers (domestic and/or industrial) and the treatments used when processing the waste (Alloway and Ayres, 1997). For example, raw sewage sludge consistently immobilised more added $^{15}\text{NO}_3^-$ than anaerobically digested sewage sludge, even when the two sludges were composted. This implied that raw sewage sludge had a higher substrate quality than anaerobically digested sewage sludge, permitting the MB to retain incoming nutrients (Epstein *et al.*, 1978).

4.2.4 THE EFFECT OF DIFFERENT MATRICES ON N MINERALISATION

After 30 days of decomposition in a soil matrix, the woolscour sludge mineralised more N than when decomposed in a sand matrix (Figure 3.23 (a)). After 50 days of decomposition, however, the amount of woolscour sludge-N mineralised in the sand and soil was similar (Figure 3.23 (b)). The higher amount of N mineralised when woolscour sludge was decomposed in soil could have been due to a priming effect, since the effect

was transient. When the fellmongery sludge was decomposed in a soil matrix there was less net-N mineralised than when decomposed in sand, this trend was consistent over 30 to 50 days of incubation (Figure 3.23). This suggested that more N were immobilised during the decomposition of fellmongery sludge in a soil matrix.

The addition of fresh organic matter to soil, such as leaf litter or sewage sludge (and presumably woolscour and fellmongery sludges), can cause a priming effect, which is a flush of N mineralisation from the soil organic matter rather than the added substrate (Swift *et al.*, 1979; Terry *et al.*, 1979). It is this flush of mineralisation that complicates the interpretation of data from soil-based microcosms; simply subtracting N mineralised from control soil-microcosms may not account for the flush. Without using a labelled woolscour and fellmongery sludge-N it is not possible to gauge the magnitude of a priming effect (if it occurred) against the effect that an environment conducive to microbial metabolism may have had on decomposition.

Pore spaces within the clay and organic matter fractions of soil provide a habitat that is more conducive to metabolic processes than ignited sand. Microsites of microbial activity develop and allow aerobic and anaerobic metabolism to occur in close proximity to each other (Killham, 1994), which in turn can permit a greater diversity in the active microbial community. There are two main benefits of using soil (rather than sand) as a matrix in microcosm studies; firstly, soil tends to have a greater water holding properties than sand, although the available-water may be greater in sand than in clay soils (Killham, 1994). Secondly, microbes will decompose both soil- and sludge-organic matter, potentially releasing trace elements from either the organic or mineral fractions of the soil. Trace elements tend to increase both net-N mineralisation and the accumulation of MB (Jenkinson, 1988).

The importance of available C to the immobilisation of fellmongery sludge-N is not unlike the decomposition of organic matter in soil. The turnover of soil organic matter may be limited by readily available C as much, if not more, than available N (Harris, 1988a). This is because soluble organic C (i.e. readily available C) is usually less than 1 % of total soil organic C (Killham, 1994). For example, 2.6 % of applied $^{15}\text{NO}_3^-$ was present as organic- ^{15}N after one week incubation and this increased to only 2.9 % after 5 weeks, which suggested that available N had not limited N immobilisation (Epstein *et al.*, 1978).

4.2.5 NUTRIENT CYCLING AND SUBSTRATE QUALITY

The effect that wooldscour and fellmongery sludges had on nutrient cycling was hypothesised to be neutral under ambient conditions (H3 in Table 2.5). This hypothesis was tested using three experiments: the co-incubation of the two sludges, the co-incubation of each sludge with flower petals and the decomposition of the sludges and wool in soil previously amended with sludge. The experimental evidence gained using these experiments indicated that fellmongery sludge tended (weakly at the replication level used here) to reduce net-N mineralisation and soil amendment led to a reduction in nutrient cycling.

The co-incubation of the wooldscour and the fellmongery sludges (i.e. in the same microcosm) indicated a weak trend towards less N mineralisation, although this was not consistent (Figure 3.24). Taken in isolation, this suggested that the sludges did not inhibit the decomposition of each other, which suggested they were neutral to nutrient cycling. However, the trend required further study, since the presence of abundant available C and N from fellmongery sludge did not enhance the decomposition of wooldscour sludge. This latter point supported the suggestion that organisms decomposing wooldscour sludge do not respond readily to fresh inputs of organic matter.

The co-incubation of the fellmongery sludge with the petals of *Genista* sp. or *Sophora tetraptera* (Kowhai), showed a weak trend of less N mineralised over what would have been expected from each substrate individually (Figure 3.25). This suggested that either, the presence of fellmongery sludge might have been interfering with net-N mineralisation, or greater immobilisation of fellmongery sludge-N occurred due to available flower petal-C. When the wooldscour sludge was mixed with flower petals, less N was mineralised than expected from *Genista* sp. petals but more from Kowhai petals. This suggested N immobilisation during co-incubation of *Genista*, since the extra N mineralised from Kowhai indicated mineralisation was not impeded by wooldscour sludge.

Therefore, based on co-incubation experiments, the low amount of N mineralised during the decomposition of the wooldscour sludge did not appear to be due to innate toxicity of the sludge. Even though the sludge was enriched in Zn and Cd, these results suggest that the metals were not inhibitory to N mineralisation. Rather, the results indicated that wooldscour and fellmongery sludges were significantly different from a substrate quality perspective.

A more robust test of the effect that the woollscour and fellmongery sludges had on nutrient cycling was gained by using soils previously amended with the respective sludges (Section 2.8.3). Significantly less N was mineralised from raw wool and both the woollscour and fellmongery sludges when decomposed in a soil that had previously been amended with woollscour or fellmongery sludges. While immobilisation of N by a microbial population better adapted to the sludge substrates is a possible explanation, the relative consistency of depressed mineralisation suggested that soil amendment had specifically compromised the activity of, at least, the zymogenous population. More N was mineralised from scoured wool, which would be consistent with enrichment for organisms with true keratinase ability (see Section 4.3.2 below).

In microbiology, enrichment culture is a standard technique used to produce a MB enriched with the specific ability to degrade a given substrate (Atlas, 1988). It was therefore reasonable to expect that soils previously treated with woollscour or fellmongery sludges would contain a greater range of organisms adapted to the decomposition of a particular sludge, than soils that had never received the waste. As this did not occur it is suggested that woollscour and fellmongery sludges were detrimental to the long-term viability of soil microorganisms. The impediment of nutrient cycling may occur when organic wastes such as woollscour, fellmongery and sewage sludges are applied to land; these sludges may contain materials inhibitory to decomposition.

Potentially, the presence of a contaminating factor, e.g. heavy metals, in woollscour sludge-treated soil had specifically reduced the zymogenic microbes. Speculatively, zymogenic organisms may be easier to extract from soil compared to autochthonous organisms, which may strongly sorb to soil particles to stabilise their cellular structure (a reasonable strategy for organisms with slow metabolic function). Inoculum from long-term woollscour waste treated soil (which would theoretically be enriched with organisms adapted to woollscour waste decomposition) did not enhance the decomposition of woollscour sludge (Table 3.14). This suggested that a zymogenic population was not strongly represented and hence not extracted when the soil inoculum was made. This is further discussed in the MB-microcosm study below (Section 4.5).

The contamination of soil with heavy metals does not explain the effect that soil amendment with fellmongery sludge had on N mineralisation (Figure 3.26), as this sludge was not particularly enriched in metals (Table 3.12). Therefore, it seems that

there was some factor(s) not determined during this work, potentially common to both sludges, responsible for the trend of reduced N mineralisation from amended soils.

4.3 CONSTRAINTS ON WOOLSCOUR SLUDGE DECOMPOSITION

It has been established that the decomposition of the wooldscour and the fellmongery sludges at ambient incubation conditions were not similar. This suggested that the two sludges were significantly different substrates and the continuation of parallel studies was not likely to address the relevant questions regarding their individual decomposition. It was therefore decided to study the N mineralisation of wooldscour sludge separately from that of fellmongery sludge. Some constraints on the mineralisation of N during the decomposition of wooldscour sludge are discussed next, and the consequences of the rapid mineralisation of fellmongery sludge-N are discussed in Section 4.4 below.

There were two relevant questions to be addressed for decomposition of the wooldscour sludge. Firstly, whether the low amount of N mineralised during decomposition were due to substrate quality or some toxic component of the sludge. This question was addressed by (i) determining whether the woolgrease fraction of wooldscour sludge was inhibitory to normal protein decomposition (H4 in Table 2.5) and (ii) the extent to which of the wool fibre in wooldscour sludge decomposed. Secondly, whether wooldscour sludge required amendment to boost net-N mineralisation during decomposition (H5 in Table 2.5). This question was studied by amending wooldscour sludge with glucose, as available C, with ammonium nitrate, as available N, and with Fe(II) as available trace metal. This latter question is important for addressing the overall potential of wooldscour sludge to act as a fertiliser in land-based disposal.

The experimental results indicated that woolgrease did not inhibit N mineralisation during the decomposition of casein. Therefore, it was concluded that woolgrease was not toxic to N mineralisation. The removal of woolgrease from wooldscour sludge appeared to denature the wool fibres; therefore it was not possible to discern the substrate quality of the wool associated with wooldscour sludge. Finally, the wooldscour sludge responded only weakly to amendment. Together these results

indicated that the principal cause of low N mineralisation during the decomposition of wooll scour sludge was the low quality of the resource for microbial metabolism.

4.3.1 THE EFFECT OF WOOLGREASE ON PROTEIN DECOMPOSITION

In 116 days of casein decomposition, 68 % i-TN was mineralised (Figure 3.27). The intimate blending of woolgrease with casein did not reduce net-N mineralisation up to and including 35 % added woolgrease (w/w; Figure 3.27). Rather than inhibiting protein decomposition, the inclusion of woolgrease at 15 % (w/w) appeared to enhance net-N mineralisation. When casein and woolgrease were mixed equally by weight, significantly less N was mineralised than from non-amended casein. Therefore, protein decomposition was not inhibited by the presence of woolgrease, up to the level in the wooll scour sludge, which supported H3 (Table 2.5), nor did woolgrease N appear to immobilise significant quantities of mineral N. This indicated that woolgrease was not directly responsible for the low amount of N mineralised during the decomposition of wooll scour sludge. Therefore, the most probable cause of low net-N mineralisation continued to be the nature of the organic N in the wool fibres, which appeared to be relatively unavailable to microbes. That is, wooll scour sludge had low substrate quality.

This finding was also reflected by the decomposition of raw wool in a soil matrix. Raw wool is surrounded by a continuous layer of woolwax (12 % woolgrease; Figure 3.29a). During decomposition, raw wool mineralised 32 % (i-TN) whereas scoured wool, which does not have a waxy coating (Figure 3.29 and Figure 3.30) mineralised 12 % (i-TN). This suggested two aspects about wool decomposition; firstly that the presence of a continuous layer of woolwax was not inhibitory to N mineralisation, and secondly that aqueous-scoured wool releases very little mineral N into the environment.

It was perhaps a little surprising that woolwax and woolgrease did not reduce N mineralisation, as the grease is potentially enriched in pesticides due to the lipophilic nature of most pesticides used in the sheep and wool industry (Greer, 1997). Therefore, the (assumed) pesticides in woolgrease did not appear to strongly inhibit non-target organisms such as bacteria and fungi, and it was concluded that if there were pesticides in the wooll scour sludge used in this experimental work, they were either sufficiently low or not specific to interfere with N mineralisation.

4.3.2 DECOMPOSITION OF THE WOOL

The treatment of woolscour sludge with petroleum ether (PE) produced two fractions, the fibre-dirt and the woolgrease. The wool fibre fraction made up 47 % of the organic fraction of the woolscour sludge and contributed essentially the entire organic N (Table 3.11). Therefore, the decomposition of woolscour sludge was strongly dependent upon the decomposition of wool.

The fibre-dirt portion of the woolscour sludge mineralised 37 % (i-TN) over 162 days, which was significantly more N than the 9 % (i-TN) maximum mineralised by woolscour sludge (Figure 3.28). The additional N that was mineralised during the decomposition of the fibre-dirt fraction of woolscour sludge was considered to have been due to denaturation of the wool fibre during PE treatment, since the woolgrease fraction had been shown not to inhibit protein decomposition or immobilisation of mineral N (above). Denaturation of the woolscour sludge-fibre by PE treatment was also indicated by the gross decrease of 2 % TN from the wool fibre-fraction of woolscour sludge (ash-free basis, Table 3.11). If denaturation were severe enough to cause the loss of N, it may have made the fibre-dirt fraction more prone to attack by general proteolytic enzymes, which would be seen as enhanced N mineralisation (Noval and Nickerson, 1959). Therefore, decomposing the N-rich fraction of woolscour sludge by itself was not, necessarily, a valid way to judge whether the low net-N mineralisation seen during woolscour sludge decomposition was due to the quality of wool fibre as a resource for microbial metabolism.

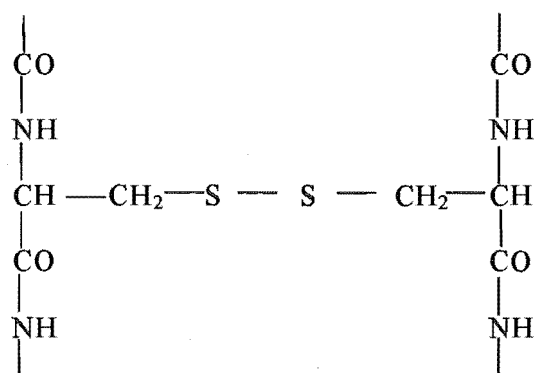
A model of native keratin degradation was required against which to determine whether the wool fibre-fraction of woolscour sludge had been denatured. Scoured wool was selected as a suitable substrate. There was strong visual similarity between scoured wool and the fibre-dirt fraction of woolscour sludge, when viewed by SEM (Figure 3.30 (b, c)). During 30 days of decomposition, scoured wool mineralised 12 % i-TN (Figure 3.26 in the control soil). After 34 days of decomposition, the fibre-dirt fraction of woolscour sludge had mineralised 17 % i-TN (Figure 3.28), which continued to increase with time of incubation. This indicated that some denaturation of the wool fibre portion of the woolscour sludge had occurred during treatment with PE.

During the decomposition of scoured wool, and presumably the fibre-dirt fraction of woolscour sludge, most of the mineralised N comes from the decomposition of non-keratinised proteinaceous material (Mathison, 1964; Stewart, 1983). Many studies using

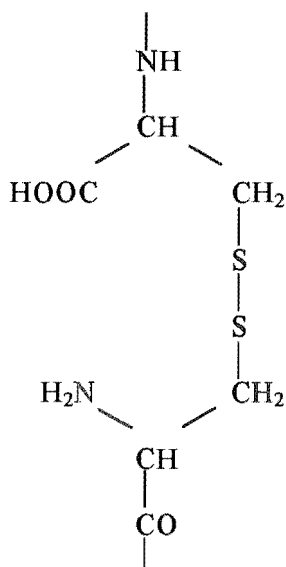
wool, hair and feathers in their natural states, have concluded they show minimal decomposition during incubation studies of less than one year (Noval and Nickerson, 1959; Benedek, 1962; Ryder, 1963; Mathison, 1964; Kunert, 1992; Malviya *et al.*, 1993a; Malviya *et al.*, 1993b). Thus, wool is often referred to as being recalcitrant.

Wool is a proteinaceous cellular fibre produced by sheep, and the principal proteins of wool, hair, feathers, horns, claws, nails and turtle shells are keratin proteins. The typical characteristics of keratin proteins are; (a) they are insoluble in water and dilute acids and bases, (b) they are resistant to non-specific proteolytic and hydrolytic enzymes, and (c) they are dissolved by substances that break the disulphide and hydrogen bonds (The Merck Index, 1976). The process of wool keratinisation involves the *de novo* synthesis of polypeptides enriched in cysteine, which is able to form disulfide bonds that can hold adjacent polypeptides together (Figure 4.43 (a)). Disulfide bonds can also form within a polypeptide, by linkage between neighbouring cysteine residues (Figure 4.43 (b)). The polypeptides arrange themselves in helices, called α -keratin, which is composed of low-S proteins (Mathison, 1964). Principally, it is the degree and alignment of the disulfide bonds that gives the exceptional chemical and biological stability to keratin materials.

Wool grows by cell division at the base of the wool follicle. The cells differentiate to become part of the cuticle (the scales of wool, which are flattened cells) or the cortex (the bulk and inner portion of the wool fibre, characterised by spindle-like cells). As the cells undergo division they are separated by intercellular material. This material is called the matrix and binds together the individual cells of wool by making a continuous layer over each cell of the fibre. The matrix, called γ -keratin, is composed of amorphous high-S proteins (Mathison, 1964). The transformation of living cells into dead keratinised cells begins about one-third of the way up the wool follicle, with one side of the fibre being keratinised before the other. This asymmetrical keratinisation contributes to the crimp of wool.



(a) Disulfide bridge between polypeptides.



(b) Disulfide bridge within a polypeptide.

Figure 4.43. The formation of disulfide bonds (a) between adjacent polypeptides and (b) within the polypeptide, from (Peters, 1963).

The wool that emerges above the skin surface is composed of fully keratinised dead cells, held together by interlocking scales and highly compressed matrix material. The wool fibres are coated with woolwax, and pushed up by the pressure of newly divided cells at the base of the wool follicle. Wool can be thought of as helices of keratinised polypeptides wrapped around each other to make cables within cables (Bamford and Elliott, 1963). The final cable (the cortex) is enclosed within a tube of keratinised cuticle cells (the scales), and tends to be bilateral in nature (Bamford and Elliott, 1963). The bilateral nature is partly due to the wool follicle that is shaped

somewhat like a golf club, with the root off-set, thus the cells are forced hard along one side of the follicle. The bilateral character of wool is important to fibre decomposition, as the ortho side, which lies on the outside-curve of the crimp is more prone to proteolytic enzymes than is the para side, i.e. the inside-curve of the crimp.

The chain of events that wool decomposition might take *in situ* can be followed *in vitro* by digesting scoured wool with enzymes isolated from *Streptomyces fradiae*, which produces potent extracellular keratinases (Brady *et al.*, 1990). For example, after 24 hours of digestion in the enzyme preparation from *S. fradiae*, the cuticle cells separate from the cortex, after 48 hours individual cells of the cortex separate, and after 4 days digestion all fibre structure had disintegrated (Kaluzewska *et al.*, 1991). Once the cuticle is breached the individual cortical cells are readily separated (Brady *et al.*, 1990). However, separated cells are not, necessarily, particularly biologically or chemically altered by this proteolytic attack (Noval and Nickerson, 1959; Lewis, 1975; Brady *et al.*, 1987; Carter *et al.*, 1988; Brady *et al.*, 1990; Kunert, 1992).

It is the magnitude of disintegration of wool fibres during decomposition (visible under light and scanning microscopes) that is potentially very misleading when assessing how much fibre has been decomposed and has minimal bearing on N mineralisation and nutrient cycling. Even scoured wool has non-keratinised proteins associated with it, about 10 % by weight, and it is thought that these non-keratinised proteins decompose before the keratinised ones (Mathison, 1964). Therefore, general proteolytic enzymes (e.g. trypsin) may completely destroy the overall integrity of a wool fibre, but solublise only 10 % of the proteins (Noval and Nickerson, 1959; Lewis, 1975; Brady *et al.*, 1987; Carter *et al.*, 1988; Brady *et al.*, 1990; Kunert, 1992).

Data on the mineralisation of wool fibre C or N are rarely presented, which makes direct comparisons to the results presented in this thesis difficult. Rather, the loss of weight of filterable material (the grade of filter is not usually mentioned) or the amount of soluble protein in the supernatant of broth cultures (obtained by filtration and then TN by Kjeldahl digestion) is used to judge the success of wool decomposition by different extracellular enzymes (Noval and Nickerson, 1959; Lewis, 1975). While the soluble proteins would, theoretically, be available to general proteolytic enzymes, the method of filtration and protein determination in the filtrate must be questioned. Individual cortical cells may pass through the filter and thus become part of the “soluble” portion of digested wool and be hydrolysed during protein determination.

When using these sort of data, considerable back-calculations are required to estimate how much of the initial wool-N had been made soluble by the digestion process and often, insufficient information is provided. In general it seems that less than 10 % of wool-N is present as mineral N after digestion with specific proteolytic enzymes, which is consistent with the N mineralised from scoured wool in the research reported in this thesis (Figure 3.26).

Accepting that different methods are used to judge the decomposition of wool, there are some important trends on keratin decomposition, which are summarised here. These points are taken from Noval and Nickerson (1959).

1. The method of keratin preparation for decomposition studies is important when the aim is to separate keratinophilic organisms from true keratinolytic organisms. Only native keratin can distinguish the two types of enzymatic suites (general proteolytic enzymes that disintegrate the fibre and specific keratinases that mineralise C and N from the keratinised proteins). Many experiments tend to use partially denatured keratin as a substrate, with the physical denaturation of keratin occurring relatively readily by most laboratory methods used for sample preparation. For example, ball milled and autoclaved wool had an average cystine content that fell from 12 % (for untreated wool) to 8 % after treatment. In addition, each of these preparative methods caused a decrease in the S content. The initial S content was 3.5 % (for untreated wool) and fell to 2.6 % for autoclaved wool and to 2.8 % for ball milled-wool. Thus, preparation of wool using these two methods can cause significant denaturation of the wool (Noval and Nickerson, 1959).
2. Ethylene oxide-treated wool fibres (for sterilisation) did not lose cystine residues, nor was the fibre more susceptible to general proteolytic enzyme attack. Before and after treatment with ethylene oxide 10 % of the wool was solubilised by trypsin (Noval and Nickerson, 1959).
3. Wool solubilisation decreased with decreasing pH. Fibre disintegration began after 5 days at pH 8.0 but not until after 14 days at pH 6.6, suggesting the enzymes that mediate dissolution of the matrix material require alkali conditions.
4. The presence of Ca and Mg salts shortened the latent period of fibre disintegration. For example, at pH 7.7 in basal salts it took 9 and 21 days for two types of wool to

begin to disintegrate, but in basal salts with the CaCl_2 and MgSO_4 doubled, it took 4 and 6 days respectively for disintegration.

5. There are some highly efficient keratinolytic microbes that can be isolated from soil, especially some *Streptomyces* species. For example, averaged over 10 different *Streptomyces* species, only 4 % of the wool had been solubilised after 8 days, and after 30 days 25 % of the wool had been solubilised. However, of the isolated *Streptomyces*, *S. fradiae* was extremely effective at solubilising wool. After 11 days incubation in basal salts, *S. fradiae* had solubilised 84 % of the dry weight of wool and half of the wool-N occurred as $\text{NH}_4^+\text{-N}$ (after hydrolysis of the filtrate). Therefore, the presence of organisms such as these in soil will explain why keratin does not accumulate in nature (Noval and Nickerson, 1959).

The disulfide bonds of wool create a strong covalently bonded substrate, with steric arrangement that impedes decomposition by non-specific enzymes. In fact, it is thought that before wool can decompose, alkaline conditions must be produced. The alkaline conditions cause some of the ionic and H-bonds to be disrupted and the fibre to swell. Once the wool fibre is in this condition, enzymes capable of penetrating the inner portions of the keratin polypeptide can specifically attack the disulfide bonds (Mathison, 1964).

Therefore, the current hypothesis for the biotic decomposition of wool is, that as well as producing proteolytic enzymes, organisms with keratinolytic capacity produce *both* alkaline conditions and reducing agents, such as sulphite, that reduce the disulfide bonds (Kunert, 1992). An intermediate in the reduction of disulfide bonds is thiosulfate, which removes two S atoms at once (Kunert, 1992; Malviya *et al.*, 1993b). This intermediate is found in exceptionally low amounts during early keratin decomposition (of human hair), but the level increases as decomposition proceeds, indicating that microbes are utilising the more readily degradable material (non-keratinised proteins) early in decomposition, in preference to keratinised protein (Malviya *et al.*, 1992).

Many organisms attributed with keratinolytic ability, and can be isolated from sites associated with sheep and birds, are probably better described as keratinophilic (Malviya *et al.*, 1992). Keratinophilic organisms mainly utilise non-keratinised proteins and decomposition by this sector of the microbial population essentially ceases when

these more readily degradable proteins are gone. For example, when a sheep dies in the field it will be decomposed by microbes that preferentially utilise proteins that are more readily available to them, such as cytoplasmic proteins, which have a higher substrate quality. The bones, wool, horns and hooves persist well after the rest of the sheep has been decomposed, indicating that these resources have a lower substrate quality compared to the rest of the sheep; a long dead sheep can, from a distance, look relatively intact. Therefore, although metabolically active organisms are intimately associated with the dead sheep most do not possess the suite of enzymes necessary to bring about the short-term decomposition of keratin.

4.3.3 THE EFFECT OF AMENDMENTS ON THE DECOMPOSITION OF WOOLSCOUR SLUDGE

The amendment of woolscour sludge with glucose, ammonium nitrate, FeCl_2 and trace metals tended to have transient and relatively minor effects on N mineralisation (Figure 3.31, Figure 3.32 and Figure 3.33). Glucose had a positive effect early in decomposition, with twice the N mineralised when glucose was added at 1 % w/w. A decrease in mineralisation at 10 % w/w glucose addition may have been due to immobilisation of N in the MB (Figure 3.31 (a)). When glucose was added after woolscour sludge had already been decomposing for 30 days there was no additional N mineralised over the unamended control. This could imply that decomposition had been completed within the short time of incubation, or that some other factor was limiting N mineralisation. Therefore, N mineralisation from woolscour sludge during the early stages of decomposition appeared to be marginally C limited, after which time the microbial population seemed to adjust to the polymeric nature of woolscour sludge-C of woolgrease and keratin.

It appeared that the amendment of woolscour sludge with ammonium nitrate decreased the amount of woolscour sludge-N mineralised during early decomposition. Like glucose, ammonium nitrate did not affect later woolscour sludge decomposition. Without labelled ammonium nitrate, it was not possible to precisely discern where the final mineral N came from, however the theoretical value was calculated from the N mineralised with no amendment and adjusted to the actual amount of woolscour sludge used. All mineral-N present on day zero was subtracted from that present on days 34

and 53. Therefore, the data (Figure 3.32) indicated that microbes preferentially used inorganic N to wooldscour sludge N during early decomposition, which is consistent with observations by other workers (Noval and Nickerson, 1959; Malviya *et al.*, 1993b). This evidence suggested that the early decomposition of wooldscour sludge was carried out by zymogenic organisms and these microbes are scavengers for inorganic N. With these organisms being potentially metabolically very active, there would be fewer resources available to the slower growing organisms. Therefore, the results of wooldscour sludge decomposing at elevated temperatures are consistent with the reduction in the number of N-scavenging organisms.

The amendment of wooldscour sludge with ferrous chloride (FeCl_2) and trace metals had a greater positive effect on decomposition than other amendments and suggested a potential requirement for iron (Figure 3.33). The Fe in wooldscour sludge, which had $7886 \text{ mg Fe kg}^{-1}$ dry sludge (Table 3.10), may have been chelated in some manner (maybe by sorption to organic material) making it relatively unavailable to microbes. Metals, especially Fe and Al, are held by organic matter, and tend to be relatively unavailable since insoluble metal-organic compounds are formed (Jenkinson, 1988); this association is highly pH dependent. As pH decreases the metals become more soluble (McLaren and Cameron, 1990), and thus more bioavailable. The pH of the microcosms during the decomposition of wooldscour sludge remained relatively neutral to slightly alkaline, indicating that the metals already chelated to organic matter were likely to remain unavailable. Since anthropogenic metals are usually considered more bioavailable (Grupe and Kuntze, 1988), the Fe^{2+} or trace metals when added to wooldscour sludge would have been initially readily available to microbes, however they would also have been prone to chelation over time.

4.4 CONSEQUENCES OF FELLMONGERY SLUDGE DECOMPOSITION

In this section one the consequences of the fellmongery sludge decomposition is addressed. The relevant question to be answered was whether mineral N was leached during the decomposition of fellmongery sludge and how much of this N was present as nitrate (H6 in Table 2.5).

Five separate experiments were used; all involved the amendment of a sand or soil matrix with fellmongery sludge at a rate equivalent to 350-kg N ha⁻¹. The effect that the quantity of water added to leaching columns had on the amount of mineral N leached was determined in experiments Leach-1 and Leach-2. The effect that ryegrass had on the amount of mineral N leached was assessed in Leach-3. These first three experiments all used a sand matrix to pack the leaching columns. Leach-4 and Leach-5 both used a soil matrix. Leach-4 assessed whether similar amounts of mineral N and nitrate were leached from soil as from sand. The long-term ability of fellmongery sludge to supply of N to crops was assessed by planting ryegrass on the spent (after 105 days of leaching treatment) soils of Leach-4. The above ground plant material was harvested and analysed. Leach-5 involved the re-amendment of the Leach-4 columns after the grass was harvested, to assess whether established ryegrass could reduce the mineral N leached.

It was found that in all cases, with or without plants, significant quantities of mineral N were leached from decomposing fellmongery sludge, with between 29 % and 39 % of the applied N leached as nitrate (Figure 3.34 and Figure 3.35). More mineral N was leached from a soil matrix than sand. The presence of ryegrass reduced the amount of mineral N leached only marginally when the grass was freshly planted, but considerably when the grass was already well established (Leach-5). Fellmongery sludge was able to provide abundant N to ryegrass, and this significantly increased the biomass production of the grass. The grass tended to contain high levels of nitrate, and in Leach-5 (re-amended soil) the grass in the amended soil all died after the 20 day post re-amendment harvest.

4.4.1 FELLMONGERY SLUDGE DECOMPOSITION USING SAND-PACKED LEACHING-MICROCOSMS

The leaching of mineral N, especially nitrate, is consistent with the aerobic decomposition of a N-rich organic substrate of medium-to-high quality (Epstein *et al.*, 1978; Mitchell *et al.*, 1978; Terry *et al.*, 1981). The amount and pattern of mineral N leached during the decomposition of fellmongery sludge on a sand matrix suggested three main aspects of fellmongery sludge decomposition. Firstly, mineralised fellmongery sludge-N was not prone to denitrification, as conditions were significantly

wetter during Leach-2 compared with Leach-1, yet similar amounts of mineral N were leached (Table 2.8 and Figure 3.35 (a, b)). Secondly, fellmongery sludge contained a consistent portion of the N that was mineralisable and leachable, irrespective of the flow-through of water (Leach-1 and Leach-2, Table 2.8). And thirdly, that while nitrification was reduced when wetter conditions prevailed (Leach-2), this reduction was due to the leaching of ammonium, the substrate for nitrification, and not to nitrification inhibition, denitrification or N immobilisation. Therefore, the ammonium leached (which to have been leached would be in excess to cation exchange sites in the soil matrix) would still be available for oxidation to nitrate later (down-stream effects). The production of nitrate away from the original source of organic N, called non-point source pollution, is of considerable concern as it can be difficult to contain and control pollution when its source remains unidentified (Alloway and Ayres, 1997).

The nitrate leached from decomposing fellmongery sludge was within the range of nitrate leached from sewage sludge applied to soil in Canada, where 15 % to 46 % of the added N was leached as nitrate (Beauchamp *et al.*, 1979). Therefore, sewage sludge and fellmongery sludge showed similar patterns of nitrate leaching, which is consistent with decomposition experiments.

The uptake of nitrate by plants is considered to be responsible for the low amount of nitrate usually present in soil water (Harris, 1988c; Wild, 1988; Killham, 1994). Therefore, it is not surprising that plants are instrumental in the reduction of nitrate leached from soil when sewage sludge and other organic waste materials are applied (Iskandar, 1978; Schalscha *et al.*, 1979; Volz and Heichel, 1979; Chopp *et al.*, 1982). Thus, the inclusion of plants to fellmongery sludge-amended sand was a reasonable way to test whether their addition reduced N leaching, as they did for sewage sludge.

Ryegrass seeds were planted on fellmongery sludge-treated sand 35 days after the columns were amended, and the grass was harvested at the termination of the experiment (Leach-3). The presence of ryegrass marginally reduced the amount of mineral N leached from the fellmongery sludge (29 % of applied N for sand-packed columns without ryegrass compared to 25 % with rye grass, Table 3.16). However, similar quantities of nitrate were leached from amended sand with no-ryegrass (20 % of applied N; Leach-2) and amended sand with ryegrass (18 % of applied N), both of which leached significantly less nitrate than Leach-1 (26 % of applied N). The lower amount of mineral N leached from amended sand with ryegrass was probably due to the

ammonium being utilised by plants, rather than leached from the soil, which was the case when the amended sand did not have plants (Leach-2). None-the-less, even with ryegrass present, fellmongery sludge still produced significant quantities of nitrate when decomposing on sand. This suggested that mineral N produced during the decomposition of fellmongery sludge were in excess to what plants could utilise in a short period of time, with the excess quickly leached at the next "rain".

4.4.2 FELLMONGERY SLUDGE DECOMPOSITION USING SOIL-PACKED LEACHING-MICROCOSMS

Having established that significant quantities of mineral N, including nitrate, were leached from decomposing fellmongery sludge on a sand matrix, the complexity of leaching-microcosms was increased by packing the columns with a 50-50 mixture of an Ilam silt loam soil and river sand (Leach-4). It was found that when the soil was amended with fellmongery sludge and leached with distilled water, more mineral N and nitrate were leached during sludge decomposition than when the sludge decomposed in the sand-packed columns. Thus, on soil-packed leaching columns, 39 % of the added fellmongery sludge-N was leached as mineral N, with 93 % of the mineral N leached as nitrate (36 % of applied N as nitrate; Table 3.16). Therefore, under optimum conditions, fellmongery sludge applied at a rate equivalent to 350-kg N ha⁻¹ had the potential to leach 103 kg mineral N ha⁻¹ from sand, largely as nitrate, and 136 kg mineral N ha⁻¹ from soil, also largely as nitrate (Table 4.25 opposite). High inputs of fertilisers (organic and inorganic) occur in many soils in temperate regions of the world, and N losses as high as 100 kg nitrate-N ha⁻¹ from arable land not uncommon (Killham, 1994).

4.4.3 RYEGRASS PRODUCTION FROM FELLMONGERY SLUDGE-AMENDED LEACHING MICROCOSMS

The production of ryegrass from fellmongery sludge-amended matrices tested H7 in Table 2.5, that similar amounts of plant biomass will be produced from a matrix amended with fellmongery sludge as from an unamended matrix. In sand-packed columns, ryegrass seeds germinated and subsequently grew on decomposing fellmongery sludge, indicating no innate toxicity to ryegrass seed germination (although

fresh fellmongery sludge inhibited the germination of cucumber seeds, Figure 6.48 (a)). In addition, fellmongery sludge appeared to provide mineral N for the production of ryegrass biomass, since significantly more ryegrass was harvested when sand was amended with fellmongery sludge than non-amended sand (Leach-3; Table 3.17).

Table 4.25. The amount of mineral N, including nitrate N (kg N ha^{-1}) leached from fellmongery sludge-amended sand and soil, at a rate equivalent to 350 kg N ha^{-1} .

Treatment	Total Mineral-N Leached	Nitrate-N Leached
	kg N ha^{-1}	
Leach-1 (sand; infrequently leached)	103	90
Leach-2 (sand; frequently leached)	101	69
Leach-3 (sand; frequently leached, plus ryegrass)	86	63
Leach-4 (soil; frequently leached)	136	126

In soil-packed columns, soil amended with fellmongery sludge and extensively leached for 105 days (Leach-4) appeared able to provide mineral N to freshly planted ryegrass. More aboveground biomass was produced when ryegrass grew on fellmongery sludge-amended soil, and the ryegrass biomass contained significantly more N (total mg of N) than that of the ryegrass biomass from the non-amended control columns (Table 3.18). However, there was no significant difference for the TN (% of dry weight) of ryegrass grown on control or amended soil. This suggested that the fellmongery-N had been used to produce biomass rather than specifically enriching leaves and stems with N.

An assumption, that at the termination of Leach-4 there was no free mineral N in the soil due to previous leaching, is reasonable since soils usually contain very little mineral N (Harris, 1988c; Wild, 1988). Yet, ryegrass grown on the fellmongery sludge-amended soil significantly benefited from the previous soil treatment, which suggested that the presence of plants was able to initiate a fresh stage of fellmongery sludge N mineralisation. It is possible that plant-derived exudates in the rhizosphere, which can

represent 5 % of photoassimilated C (Killham, 1994), provided available C to the soil MB, which in turn allowed a renewal of fellmongery sludge decomposition.

4.4.4 NITRATE IN THE LEACHATE FROM FELLMONGERY SLUDGE-AMENDED LEACHING MICROCOSMS

The USA Environmental Protection Agency (EPA) has set an upper limit of 10-mg $\text{NO}_3^- \text{N L}^{-1}$ (10 ppm) for potable water, which has become a bench mark for pollution assessment (Kingery *et al.*, 1994), although the European Commission has a 50 ppm limit for nitrate (Killham, 1994). In the work here, the upper EPA limit for nitrate in drinking water was exceeded in all leaching experiments within 10-30 days post-amendment with fellmongery sludge, and remained above the limit for a further 45 to 80 days (see Figure 4.44 opposite). Nitrate can enter the food chain through its accumulation by plants or in drinking water. Excess nitrate in the diet, especially of children, is a concern of health authorities; the reduction of nitrate, to nitrite, in the stomach is thought to be the cause of methemoglobinaemia, where haemoglobin is oxidised making it unable to carry oxygen. This is a particular problem where potable water (and trough water for animals) is drawn from wells that receive recharge from land where fertilisers, such as sewage wastewater, are heavily applied (Schalscha *et al.*, 1979).

Plants accumulate N by excessive protein synthesis during the vegetative stage of growth, which is strongly promoted by nitrate, thus producing more and bigger leaves (Wild, 1988). However, the carbohydrate synthesised during this time is proportional to the photosynthetic capacity of the plant, and if much of the C assimilate is being diverted into protein synthesis little is left for incorporation into cell wall material (Wild, 1988). If the plant is growing new leaves rapidly, the cell walls of these leaves may be thin. Thin cell walls has a flow-on effect to the physiology of the plant, such as increased susceptibility to the environmental stresses of frost, drought and pests. The crop may be damaged by insufficient strength to stand straight (e.g. cereal crops), or the reduction in carbohydrate content of crops such as sugar cane, which is grown for its carbohydrate content. Water stress may occur as the plant tries to support the large leaf biomass, and usually a leafy biomass delays crop maturity, which may be a problem for grain and fruit crops (Wild, 1988; Troeh and Thompson, 1993). Therefore, plants that

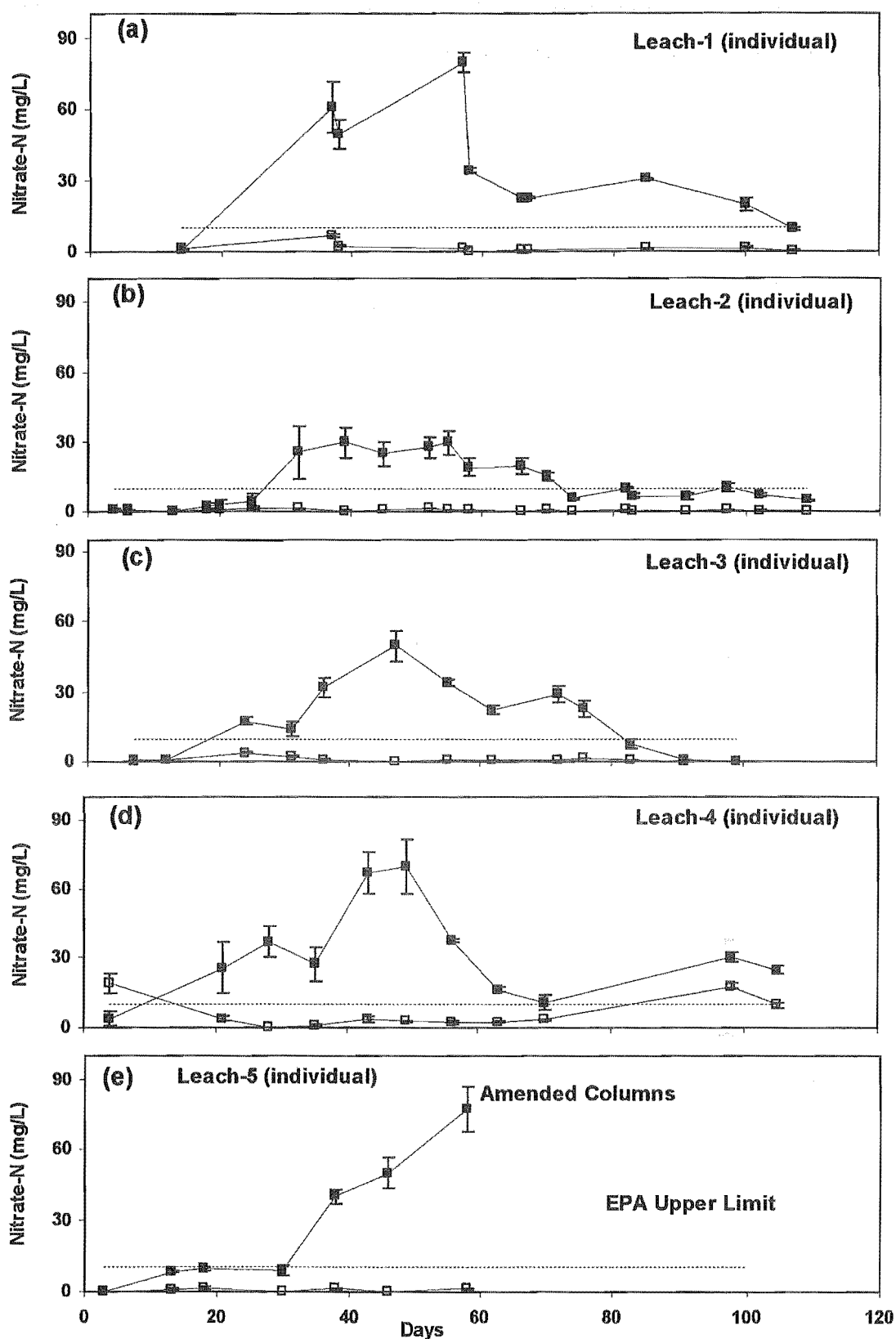


Figure 4.44. Nitrate leached from fellmongery sludge-amended (■) and control (□) columns presented in relation to the EPA potable water upper limit (10 mg NO₃-N L⁻¹). Amendment was at a rate equivalent to 350-kg N ha⁻¹. (a) Leach-1, (b) Leach-2, (c) Leach-3, (d) Leach-4, and (e) Leach-5. Mean (± SE), n=3.

accumulate N out of proportion to the C fixed will have an elevated TN as a percent of dry weight. Based on this, the grass produced from Leach-4 had utilised available N in proportion to the amount of C fixed since the amount of N per mg plant biomass was the same for control and amended soil plants (Table 3.18).

4.4.5 FELLMONGERY SLUDGE-AMENDED SOIL-PACKED AND RYEGRASS PLANTED LEACHING MICROCOSMS (LEACH-5)

Ryegrass growing in the soil of Leach-5 responded well to a fresh application of fellmongery sludge (at a rate equivalent to 350-kg N ha⁻¹), producing 375 mg (dw) of grass in 20 days (Table 3.19). In addition, the ryegrass grown with fresh fellmongery sludge contained 13 times more N, and four times more nitrate than the ryegrass from the non-amended soil (control columns). This indicated that, as well as promoting the production of more biomass, freshly decomposing fellmongery sludge promoted significant accumulation of N and nitrate in the plant tissue. This could cause problems later for cattle consuming the grass (nitrate poisoning) and plant tissue with thin cell walls may be susceptible to frost, wind etc (Wild, 1988).

After the ryegrass was harvested (20 days post-amendment of Leach-5), no grass regrew on the fellmongery sludge-amended soil. This was surprising, given that ryegrass seeds germinated and grass grew readily on fellmongery sludge-amended sand in Leach-3. The reason(s) for the failure of ryegrass after 20 days remains unresolved, as the ryegrass on the control columns re-grew, indicating that the method of harvest was not responsible for the failure of the grass in amended soil to re-establish. A possible explanation is presented.

Prior to ryegrass harvest, high transpiration and photosynthesis may have allowed the ryegrass to cope with the osmotic stress and high energy demands of elevated nitrate in the soil water (Raven *et al.*, 1986). Energy demand is high during immobilisation of N, as energy is required to reduce nitrate to ammonium, which is then incorporated into amino acids and organic compounds. The organic N is then translocated away from the roots to the growing regions of the plant (Raven *et al.*, 1986). Since the roots of the ryegrass also remained in the fellmongery sludge-amended soil after harvest of the above ground material, nitrate may have accumulated in the rhizosphere to a level toxic

to the plant tissue, thus preventing the grass from producing new leaves. Root failure may also have occurred, due partially to nitrate toxicity and partly to the collapse of conducting tissue (Wild, 1988). Failure of root function has also been associated with soil depleted in soil oxygen and exposed to toxic products of anaerobic metabolism (Killham, 1994). Such anaerobic conditions may have developed during the enhanced microbial activity during fellmongery sludge decomposition, as any toxic products would have been washed into the rhizosphere during the leaching treatment. A combination of these situations would have led to plant death. Whatever the reason for the ryegrass dying in the fellmongery sludge-amended soil during Leach-5, the presence of well established (rather than freshly sown) ryegrass had significantly reduced mineral N (specifically nitrate) leached during the early stages of fellmongery sludge decomposition (Figure 3.38 and Figure 4.44). From the time of grass harvest (day 20) to the termination of the experiment (day 57), approximately 14 % of the i-TN was leached as nitrate in only 37 days.

Extrapolation of these microcosm results to field conditions may not be completely valid, since the miniaturisation of a microcosm may distort the magnitude of leaching. However, the results strongly indicate the potential for significant amounts of nitrate to be leached from surface-applied fellmongery sludge, and indicate that extensive long-term field monitoring should be instigated wherever fellmongery sludge is disposed of on land. If the magnitude of nitrate leached under field conditions approaches that of the microcosm experiments, then the sustainability of land-spreading as a waste disposal option should be re-examined.

4.5 THE MICROBIAL BIOMASS OF WOOLSCOUR AND FELLMONGERY SLUDGE-AMENDED SOIL

One of the consequences of the amendment of soil with woolscour or fellmongery sludge was the reduction in the amount of N mineralised from added substrates such as raw wool, woolscour sludge and fellmongery sludge (Figure 3.26). This implied that the MB was not able to mineralise N as well as it had prior to soil amendment. Therefore, the relevant question to be answered was whether the MB had been reduced by soil amendment with the woolscour and fellmongery sludges (H8 in Table 2.5). This question was addressed by estimating the MB of soils amended with the sludges. The

results from a microcosm-scale experiment indicated that one-year after soil amendment the quantity of the soil MB had fallen dramatically, to levels below detection by the fumigation-extraction method. A similar trend, although not as extensive, was seen when soils were amended at the field-scale. In both cases the amendment of soil caused an increase in the TN of soil.

4.5.1 MICROBIAL BIOMASS OF SOIL AMENDED WITH WOOLSCOUR AND FELLMONGERY SLUDGES: MICROCOSM STUDY

The amendment of soil with fellmongery sludge caused a significant and immediate increase in the MB (Figure 3.25), possibly due to resuscitation of freeze-dried organisms during the post-sieving incubation stage of MB determination (Brookes *et al.*, 1985b). After 50 days of sludge decomposition, the amended soils had significantly more MB than the non-amended controls (% of soil N; Figure 3.39). This indicated that, firstly, presenting MB-N data as a percentage of soil N was sensitive to variation in the relationship between MB and soil organic reserves (Jenkinson and Ladd, 1981; Sparling, 1997). Secondly, after 50 days of decomposition, amendment of soil with woolscour and fellmongery sludges had had a positive effect on the MB, which is consistent with the benefits of applying organic substrates that contribute both C and N to the soil (Jenkinson and Ladd, 1981). This result also suggested that the MB responsible for decomposing woolscour and fellmongery sludges during the usual 30 to 50 days of incubation in the decomposition experiments (Section 3.3) was not compromised metabolically. Thus, the mineralisation data in Sections 3.3 to 3.4 are considered a fair representation of the potential of these two sludges to decompose and indicate that the actual turnover of the sludges is slightly higher than net-N mineralisation shows, since there has been immobilisation of sludge nutrients in the MB.

After 365 days decomposition, however, the fumigated amended-soils had similar amounts of KCl-extractable TN as the non-fumigated amended-soils, suggesting that the MB was below detection level by the fumigation-extraction technique (Figure 3.39). Therefore, amendment of soil with woolscour and fellmongery sludges had had a long-term and deleterious effect on the MB. The microcosm system was not considered responsible for the decline in the MB, for there was no significant difference in MB-N

of control soil on day zero, day 50 and day 365 ($p = 0.1285$). This may explain the trend of reduced net-N mineralisation over long-term decomposition experiments using wooldscour sludge (Figure 3.19), which when interpreted in conjunction with MB supports the conclusion that the presence of wooldscour sludge compromises the metabolic functions of microorganisms.

The explanation for the dramatic decrease in the quantity of the soil MB was not clear and suggested a factor(s), potentially common to both sludges, was inhibiting microbial survival. One candidate that could have decreased the MB was the presence of heavy metals in the sludges. The relative concentration of Zn and Cd in the wooldscour and fellmongery sludges may have effectively increased as readily available organic matter was utilised. The decline in MB could have, therefore, been due to the presence of heavy metals in the wooldscour and fellmongery sludges. This scenario suggests a chronic impact of heavy metals upon soil microorganisms (Wardle, 1992).

Sewage sludge is typically contaminated with heavy metals. For example a low-metal sewage sludge had Zn at 1354 ppm and Cd at 8.4 ppm (Chander *et al.*, 1995), and a high-metal sewage sludge had Zn at 6000 ppm and Cd at 40 ppm (Fliebbach *et al.*, 1994). Soils amended with high-metal sewage sludge have shown a decline in the quantity of MB, however soils amended with low-metal sludges tend to benefit from sludge addition, as shown by an increase in the MB (Fliebbach *et al.*, 1994; Chander *et al.*, 1995). The level of metals, especially Zn, associated with sewage sludge was higher than that of the wooldscour sludge, although the level of Cd in the wooldscour sludge was similar to the low-metal sewage sludge. However, based on a sewage sludge model, the strong negative effect seen on the quantity of the soil MB would not have been predicted due to soil amendment with wooldscour sludge, since the level of metals were well short of those in the high-metal sewage sludge. In addition, since fellmongery sludge was not particularly contaminated with heavy metals and amendment of soil with this sludge also caused a catastrophic effect on the soil MB, it is suggested that some other factor(s) was negatively affecting microbial survival. The presence of pesticide residues may have caused a decline in the MB of sludge-amended soils. The effect of pesticides in decreasing the MB is likely to be on the function of soil fauna and their role in the turnover of the MB, since fungi and bacteria are not necessarily strongly affected by non-target pesticides (Wardle, 1992).

Nutrient cycling is essential to the continuation of life on Earth, and microorganisms are responsible for essentially all mineralisation processes. Any alteration in the microbial habitat that decreases mineralisation will, eventually, have a negative impact upon the health and function of the soil (Sparling, 1997). The quantity of MB can be used as a tool to assess the impact of land-based waste disposal. The MB is sensitive to changes in soil management, and these changes are reflected in the MB before they are observed in crop productivity (Wardle, 1992). A soil with a severely depleted MB will, in turn, have a negative effect on long-term nutrient cycling (Jenkinson and Ladd, 1981; Brookes *et al.*, 1986; Wardle, 1992). Most of the mineralisation studies discussed above (Section 4.1.3) were carried out during the period of time before the woolscour and fellmongery sludges negatively affected the quantity of MB (up to about 50 days). This may explain why only a weak effect was seen for nutrient cycling when flower petals and fellmongery sludge were mixed with woolscour sludge, since they were incubated for 30 to 50 days (Figure 3.24 and Figure 3.25). The stronger effect of woolscour and fellmongery sludges on nutrient cycling was seen when the sludges were decomposed in soils amended 10 months previously (Figure 3.26). This strongly indicates that future mineralisation studies need to include soils that have received long-term woolscour and fellmongery sludge amendment in order to assess the sustainability of land-based disposal and its effect on nutrient cycling.

4.5.2 MICROBIAL BIOMASS OF SOIL AMENDED WITH WOOLSCOUR AND FELLMONGERY SLUDGES: FIELD STUDY

Similar to the MB-microcosm study, the TN of woolscour and fellmongery sludge-amended soils on the field scale was higher than that of the control soil, although only woolscour sludge-amended soil was significantly higher (at $\alpha < 0.05$, Table 3.21). In the field study, the woolscour sludge-amended soil had 31 % more TN than the control soil, whereas in the microcosm-study soil amended with woolscour sludge had 16 % more TN than the control (Table 3.21). This suggested a greater build up in soil N when woolscour sludge decomposed under field condition and with plants present than seen in the MB-microcosm study. The additional N may be due to less woolscour sludge being mineralised, however it could equally be due to fine root material remaining in the soil during TN determination.

The amendment of soil with woolscour and fellmongery sludges, did not significantly alter the organic matter or moisture contents of the soil, although there was a trend that soil amendment increased both properties compared to the control soil (Table 3.21). If the one-time application of woolscour and fellmongery sludges had increased the organic matter of soil, even though non-significantly in this study, it suggested that further applications would cause an accumulation of organic matter. Whilst an increase in organic matter is usually associated with improved soil conditions (Swift *et al.*, 1979), when it is brought about by the addition of sludges, to soils otherwise sufficient in organic matter, it can be a sign of decreased nutrient cycling (Chaney *et al.*, 1978).

The level of mineral N in the control and amended soils was not significantly different, although slightly more mineral N was present in the fellmongery sludge-amended soil (Table 3.21). Essentially all mineral N was present as nitrate, which indicated that soil amendment had not specifically affected nitrifying organisms. The lower pH of fellmongery sludge-amended soil would be the expected result of nitrification (due to the release of H^+ ions during oxidation of ammonium); nitrification readily occurred during fellmongery sludge decomposition (Figure 3.35).

The amendment of soil with woolscour and fellmongery sludges tended to cause a decrease in MB-N (% of soil N, Table 3.21), which was consistent with (although considerably weaker than) results from the MB-microcosm study above. The pH of fellmongery sludge-amended soil (6.7) was only slightly lower than both the control (7.0) and woolscour sludge-amended (7.1) soils, and not considered to be biologically different to the other soils even though statistically it was lower (Table 3.21). It is extremely unlikely that the slightly lower pH (0.4 of a unit) of fellmongery sludge-amended soil made metals associated with the fellmongery sludge more mobile under field conditions than that of the metals associated with woolscour sludge. Therefore the trend of decreasing MB (% of soil N) does not appear to be due to metal toxicity and supports a suggestion that some other factor, potentially similar in both sludges, was negatively affecting the MB.

It is probable that the presence of plants in the field study provided a constant renewal of available C and N to the rhizosphere (*via* root exudates) and the soil system as a whole through plant detritus (Wardle, 1992; Killham, 1994). This may have permitted the MB to tolerate stresses imposed by the sludges (pesticides, metals, grease,

recalcitrant C and N etc.) better than seen in the microcosm study. In addition, the plants may have immobilised metals (Alloway and Ayres, 1997) thus reducing the effective available-metal concentration. Metals associated with the sludges could have been leached from the top 20-cm of the soil profile, reducing their inhibitory effects on the MB. However, at the relatively neutral soil pH of amended soils (Table 3.21) metals associated with the sludges were unlikely to be particularly mobile (Carey, 1994; Sawhney *et al.*, 1994).

There are no published investigations concerning the effect(s) of woolscour and fellmongery sludges on the soil MB. Hence, sewage sludge is again used as a suitable model against which to judge the effect that soil amendment with woolscour and fellmongery sludges may have on the MB. The comparison includes soils that received organic fertilisers, including farmyard manure and sewage sludge, and inorganic fertilisers for 31 years (Witter *et al.*, 1993). Witter *et al.* (1993) showed that the amendment of plant-free soil (plants were removed regularly by hand) with inorganic fertiliser did not increase soil N, however, when the soil included plants, both organic matter and soil N increased (Table 4.26 opposite). They found that the amendment of soil with organic fertilisers significantly increased soil N levels (Table 4.26). A similar trend was seen during the current study when soil was amended with woolscour and fellmongery sludge (Table 3.21), and is consistent with evidence that organic amendments to soil generally boosts soil N (Wild, 1988).

In the study by Witter *et al.* (1993), the most obvious influence of organic amendment of soil came from addition of farmyard manure (Table 4.26). This fertiliser increased the absolute level of MB-N ($\mu\text{g g}^{-1}$ soil) to 150 % more than the cropped but unfertilised soil, and significantly increased (by 109 %) the proportion of the soil N immobilised in the MB, MB-N (% of soil N; Table 4.26). Therefore, the long-term amendment of soil with farmyard manure was considerably better for the MB than long-term amendment with sewage sludge (Table 4.26), indicating that sewage sludge had had a negative effect on the soil MB (Witter *et al.*, 1993). Soil amended with sewage sludge increased the absolute MB-N by 10 %, but showed a 41 % decrease in the N immobilised in the MB (% of soil N).

The one-time application of woolscour and fellmongery sludge had had a similar impact on the soil MB as sewage sludge in the above example; there was a 10 % and 2 % increase in the absolute MB-N for woolscour and fellmongery sludge-amended soils,

respectively. However, a 20 % and 17 % decrease in the N immobilised in the MB (MB-N as % of soil N) for woollscour and fellmongery sludge-amended soils in the field, respectively, occurred (Table 3.21). Therefore, woollscour and fellmongery sludge-amended soils showed similar trends to those seen for the sewage sludge-amended soils (Table 4.26, Witter *et al.*, 1993); the absolute MB-N was slightly elevated but the soil N-immobilised in microbial tissue was reduced when soil was amended. Together with decomposition results, the MB studies indicated that sewage sludge was a reasonable model for woollscour and fellmongery sludges, however, the model was stronger for prediction of fellmongery sludge interactions than woollscour sludge.

Table 4.26. Soils amended with organic (4 t ash-free organic matter ha⁻¹ yr⁻¹) and inorganic fertilisers (80 kg N ha⁻¹ y⁻¹) over a period of 31 years. MB was determined by fumigation-incubation technique (k_N of 0.57). From (Witter *et al.*, 1993).

Soil Treatment	C:N ratio	Soil TN (% dw)	MB-N ($\mu\text{g g}^{-1}$ soil)	MB-N (% of TN)
Fellow (unfertilised)	7.8	0.13	35	2.7
Cropped (unfertilised)	7.4	0.16	48	3.2
Cropped Ca(NO ₃) ₂	8.2	0.16	61	3.8
Cropped (NH ₄) ₂ SO ₄	8.1	0.17	19	1.1
Cropped Farmyard manure	9.3	0.21	120	6.7
Sewage sludge	9.5	0.28	53	1.9

4.5.3 MICROBIAL BIOMASS OF SOIL AMENDED WITH WOOLSCOUR AND FELLMONGERY SLUDGES: GLASSHOUSE STUDY

In general, and assuming adequate energy is available, the MB has a greater turnover at temperatures between 20 and 28 °C than at lower temperatures of 4 to 12 °C, indicating that nutrients immobilised within the MB will be re-mineralised quicker at warmer temperatures (Nicolardot *et al.*, 1994). The re-mineralisation of N permits the pool of N-limited soil microbes to utilise organic matter of a lower substrate quality *via* decomposition of dead microbes, which had already utilised resources of higher substrate quality (Wardle, 1992). Therefore, the hypothesis was tested that the MB of

woolscour and fellmongery sludge-amended and non-amended soils will respond in a similar manner when the soil is incubated under ambient conditions (H9 in Table 2.5).

The glasshouse conditions (18 °C and regular watering) used during the incubation of soils that had been amended with woolscour and fellmongery sludges in the field and incubated there for five months, were conditions closer to those for optimum turnover of organic matter than field conditions. Similar to the MB from the microcosm and field studies, woolscour and fellmongery sludge-amended soils incubated in the glasshouse for five months, showed a decrease in N immobilised in the MB (% of soil N, Table 3.14). Therefore, the results from these MB studies have consistently indicated that the amendment of soil with woolscour and fellmongery sludges had a negative effect on the soil MB, and suggested that some factor was limiting the ability of the soil MB to immobilise N.

The MB of soils (amended and control) incubated in the glasshouse contained significantly more MB (% of soil N) than their field soil counterparts (Table 3.16 and Figure 3.28). The glasshouse-incubated control soil had 46 % more, the woolscour sludge-amended soil 75 % more, and the fellmongery sludge-amended soil 60 % more MB-N (% of soil N) than the field soils (Figure 3.41). This indicated that although the soil MB had been reduced due to amendment in the field, the organisms that survived were metabolically competent, suggesting that a change in soil management of woolscour and fellmongery sludge-amended soils would directly affected the MB.

An index such as the response of the MB to altered incubation conditions could be a useful tool to indicate soils that are likely to respond well to bioremediation. Such a laboratory-based biological tool would permit useful data to be accumulated considerably faster than when measuring effects of management in the field, where seasonal fluctuations mean that determinations need to be made at the same time of the year. The idea of using the MB as an indicator of soil health is currently topical (Sparling, 1997). The use of a microbial quotient (the ratio of microbial-C-to-soil-C) reflects the sensitivity of the MB to fluctuations in its habitat, and permits the normalisation of data from soils that may contain different amounts of soil organic matter (Sparling, 1997). Therefore, the equivalent to the microbial quotient except using N as the parameter determined also seems sensitive to changes in soil management and the quantity of the soil MB.

4.5.4 PLANT MATERIAL HARVESTED FROM WOOLSCOUR AND FELLMONGERY SLUDGE-AMENDED SOILS: GLASSHOUSE STUDY

The above ground plant material harvested from woolsour and fellmongery sludge-amended soils (incubated under glasshouse conditions; Table 3.24), were not significantly different for plant biomass or N content as plants harvested from the control soil. The root biomass was not determined during this work, partly because in future long-term field studies it would be expected that the sludges may be applied to pasture, which would be grazed but the roots retained in the soil to grow new leaves. There were some noteworthy trends seen for the plant material harvested from the glasshouse-incubated soils.

Firstly, the fellmongery sludge-amended soil produced more above ground biomass, which contained more N (ha^{-1}) than the control or woolsour sludge-amended soils, although the difference was non-significant. The plant material harvested from fellmongery sludge-amended soil contained 19 % more N (kg N ha^{-1} basis) than plants grown on the non-amended control soil. Plants grown on the woolsour sludge-amended soil contained 16 % less N (kg ha^{-1}) than the control. The fellmongery sludge-amended soil had received three-times the amount of sludge-N (195 kg N ha^{-1}) than the woolsour sludge-amended soil (at 60 kg N ha^{-1}), and in mineralisation studies had mineralised more N than woolsour sludge. It was therefore surprising that the plant biomass harvested had not responded more strongly to amendment of soil with fellmongery sludge. This may indicate that, as shown in the N mineralisation experiments (Section 3.4.6), the amendment of soil with fellmongery sludge had compromised nutrient cycling.

Secondly, the woolsour sludge-amended soil was lower in all parameters measured for plant production (Table 3.23). This non-significant trend was consistent with the mineralisation studies that had showed woolsour sludge released less N than fellmongery sludge.

These results are consistent with the microcosm studies that indicated that fellmongery sludge has the potential to contribute N to plants, even if the trend was non-significant in the field study. The results were also consistent with the microcosm studies for woolsour sludge, which showed that less N was mineralised and therefore available to plants. Together, these data suggested that the application of woolsour

sludge at a rate that contributed equivalent N to fellmongery sludge would not met the N requirements of the growing plants.

5 CASE STUDY ONE: MICROBIAL BIOMASS OF SOIL AMENDED LONG-TERM WITH WOOLSCOUR EFFLUENTS

5.1 INTRODUCTION

There are soils around New Zealand that have received woolscouring wastes for many years and, thus, would be appropriate to use when determining the effect(s) that land-based disposal has had on soil properties, especially the soil MB. However, managers of such woolscouring plants are somewhat reluctant to participate in University-based research, the findings of which could be widely disseminated.

In 1995, I was offered an unique opportunity to sample soils that had been treated with woolscour effluent for approximately 23 years, *and* be allowed to present the results of MB estimates as a case study in this thesis. The farm, from which the soil samples were obtained, functioned primarily as a waste disposal system. It consisted of a large area of land, near a river, to which woolscour wastes were applied on an essentially daily basis.

In 1995, the woolscour plant had an output of 80,000 bales of scoured wool. However, in the 1980s a previous management had encountered extreme difficulties disposing of the effluent onto *only* the woolscour farm, when scoured wool production was increased from 50,000 bales to 100,000 per year; large areas of the farm became waterlogged and completely unproductive (communication from WRONZ). All wastes from the wool scour are currently disposed of over an area of approximately 1600 ha, which includes all neighbouring farms. Sludges are applied to paddocks (*via* tankers) and washed into the soil with flowdown, usually diluted with rinse water. There is a history of burying heavier sludges, although this has essentially stopped under the current management. No fertiliser has been used on the farm for 30 years, other than that provided by woolscour effluent. The new management has succeeded in regaining productivity of most paddocks that were damaged in the 1980s, and is now running about 500 head of sheep and 30 head of cattle, which was considered by the farm manager to be low for an irrigated farm.

Soil sampling was in the drought-prone month of January, however most of the paddocks at the woolscour farm were lush with grass and green compared to the region

as a whole. The lush grass growth may be the result of (a) the water in the woolscour effluent, (b) the organic and inorganic material in the woolscour effluent, or (c) a combination of the two. Since the soil of the woolscour farm was a free-draining Waimakariri stony sandy loam (Campbell *et al.*, 1980), it can be assumed that, apart from a few low-lying areas, the paddocks would be well drained. Therefore, in a drought-prone region, the water fraction of woolscour wastes would promote grass growth and immobilisation of soil N in the MB (Harris, 1988b). However, earlier in this thesis, amendment of soil with woolscour sludge reduced the ability of the soil MB to mineralise and immobilise added N (Figure 3.26 and Figure 3.39 b), which suggested that an estimate of the MB was relevant to discern the effect of woolscour effluent on soil. This case study is an example of applied research, and although access to the farm was on the conditions that a WRONZ staff member was present during soil sampling and the farm location remained confidential, it serves to indicate a real situation of woolscour waste disposal in New Zealand.

5.2 MATERIALS AND METHODS

The general layout of the area sampled is shown in Figure 5.45. The soil was a Waimakariri stony sandy loam (Campbell *et al.*, 1980). Six sites were sampled using a 2-cm diameter auger to a depth of 20-cm (Table 5.27). Samples were placed in plastic zip-lock bags while in the field, and once in the laboratory, stones and visible vegetation were removed. Soils were then sieved to <2 mm and stored in clean zip-lock plastic bags at 5 °C until analysis. Soil samples are referred to as “woolscour-farm soils”.

Organic matter was determined as in Section 2.3.3 and TN as in Section 2.3.6. Analyses were in duplicate and are reported on an oven dry weight basis.

Microbial biomass was determined using the fumigation-extraction method (Brookes *et al.*, 1985b) (Section 2.8.1), except that ethanol was not removed from the chloroform.

Table 5.27. Summary of the paddocks sampled for microbial biomass. The paddock sampled (P) and is followed by a site number (S), where appropriate.

Paddock	Description of Site
P2	Grazed by sheep. No sludge or flow-down had been applied for about three months. All irrigation had been by dyke flooding, using rinse water only.
P5	Grazed by sheep. Considered the "worst" paddock on the farm. Large amounts of sludge have been buried in P ₅ for at least 23 years. There was evidence of boggy patches in several sites on this paddock. Soil samples were taken well away from boggy patches. Surplus sludge was still applied, usually in winter, rinsed in with flow-down and rinse water by boarder dyke flooding or spray irrigation.
P7	Grazed by sheep and cattle. It had been treated with sludge two weeks (and in some places two days) before sampling. The sludge had been applied from a tanker driven over the paddock. The applied sludge was then rinsed into the soil with flow-down diluted with rinse water by spray irrigation. There were scattered patches of sludge still on the surface, as well as many patches where the grass had turned brown and was completely shrivelled. The paddock had been ploughed and sown in grass two years previously.
P7S1	This site had sludge applied two weeks previously. It was sampled where there was no visible crust of sludge and away from brown patches of grass.
P7S2	This site had been treated with sludge two days before sampling and dried crusts of sludge were present on the surface. It was not possible to sample without including sludge, however this tended to stick to the grass and could be removed by the sieving process at the laboratory.
P7S3	This site was sampled because it was the low point on the paddock and had poor drainage when the paddock was irrigated. It represented a site of probable soil stress due periodic water logging.
P10	This paddock had been treated with sludge about a month before sampling and was very lush in rye grass and currently grazed by 30 head of cattle.

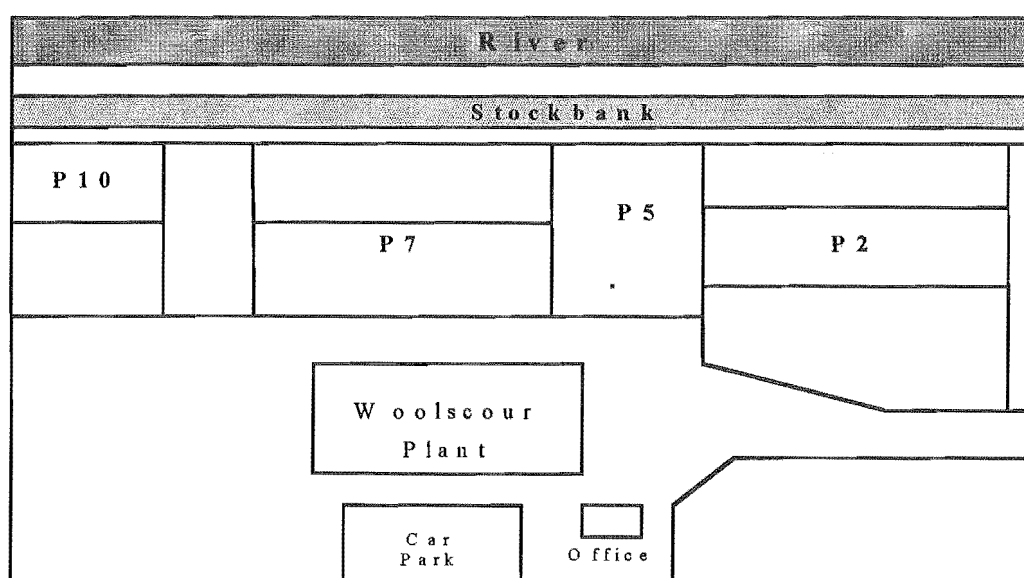


Figure 5.45. General layout of the paddocks with respect to the scour plant. Not to scale.

5.3 RESULTS AND DISCUSSION

The woolscour farm and surrounding farms had received woolscour wastes recently, which meant there were no untreated or “control” soils available. Therefore, comparisons can only be made between the different treatments used on woolscour farm-paddocks and information provided by the management. The net effect of woolscour wastes on soil cannot be made, although it is possible to make inferences. To compensate somewhat for the lack of a control soil, two sets of data have been used in order to examine the effects of woolscour waste on these soils. Firstly, a series of agricultural soils on the Park Grass permanent grassland (since the woolscour-farm soils were pastures) experiment at Rothamsted are used (Brookes *et al.*, 1985b) and secondly, the soil amended with woolscour sludge in the field experiment described in Section 2.8.3.

5.3.1 SOIL ORGANIC MATTER AND NITROGEN

Most mineral soils in New Zealand have topsoil organic matter levels ranging from 3 to 20 % (McLaren and Cameron, 1990). With the exception of P5S1, the organic matter of the woolscour farm-soils ranged from 4 to 6 % (Table 5.28), which was similar to a study 15 years previously (Campbell *et al.*, 1980). P5S1 was not sampled in the 1980 study, and in the current work had an organic matter level of 12 % (Table 5.28), which was considerably elevated compared to the other woolscour-farm paddocks. Hence, the practice of burying large quantities of wastes in this paddock, for at least 23 years, appeared responsible for the increased level organic matter.

The woolscour sludge used in the decomposition and MB experiments above (Section 2.2.1) was the “worst woolscour sludge” currently produced in New Zealand (personal communication from WRONZ). As such, the experimental-woolscour sludge may be similar to sludge applied to, and buried in, P5S1 in the past. The experimental-woolscour sludge contained high levels of woolgrease, wool fibre, Cd and Zn (Table 3.9 and Table 3.12). The long-term application of similar wastes to P5S1 would be expected to reduce the decomposition of organic matter (as seen in Figure 3.26), thus causing the accumulation of organic matter in the soil. This is also suggested by comparison with the other woolscour-farm soils, which had lower levels of organic matter than P5S1 (Table 5.28). A similar finding was reported after repeated

applications of sewage sludge contaminated agricultural land with heavy metals (Fliebbach *et al.*, 1994).

Table 5.28. The pH, organic matter and TN of soils (0-20 cm depth) collected from a farm attached to a wool scouring plant. Means are reported on an oven dry weight basis, $n = 2$.

Site	pH	Organic Matter (% dw)	Soil TN (% dw)
P2S2	8.8	4.9	0.18
P5S1	6.3	11.7	0.69
P7S1	7.2	4.0	0.15
P7S2	7.8	4.3	0.16
P7S3	7.8	5.2	0.20
P10S1	6.9	6.0	0.24

Agricultural soils typically contain 0.1 to 0.3 % N in the top 15 cm (McLaren and Cameron, 1990) and the soils from the woollscour farm fell within this range, except for P5S1, which had 0.7 % N (Table 5.28). The high level of N in P5S1 is consistent with the long-term practice of dumping woollscour sludges onto (and into) this soil, as a similar results above was found when Ilam soil was amended with woollscour sludge (Table 3.20 and Table 3.21). There was relatively little wool fibre in the sieved woollscour waste amended-soil, suggesting major fibre disintegration over time, which had not, apparently, been accompanied by significant N mineralisation (Figure 3.19 and Section 4.3.2).

Agricultural soils that have high organic matter *and* high TN are usually considered fertile and productive, *i.e.* a soil that can provide adequate amounts of water and nutrients as well as physically support organisms and promote plant growth (Doran and Safley, 1997). According to the manager, P5 was an unproductive paddock that was unable to support prolonged grazing. This suggested that, in this case, high organic matter and high TN were deceptive. The practice of excessive application of wastes to paddocks (including P2, P7 and P10) had stopped ten years previously. Hence, the results suggest that the soils of P2, P7 and P10 had not been irreversibly damaged, since they do not stand out as being different to agricultural soils for organic matter and N (Broadbent, 1965; McLaren and Cameron, 1990).

5.3.2 MICROBIAL BIOMASS

Microbial biomass is a key factor controlling C and N mineralisation in soil and any physical, chemical or climatic factors that affect the MB will in turn affect mineralisation processes (Jenkinson, 1988). Soil MB and N availability are highly correlated (Franzluebbers *et al.*, 1995), making MB a sensitive indicator to assess the impact that land-based disposal of woolscouring wastes has had on the soil.

Soils from the woolscour farm had absolute MB-N ranging from a low of 50 $\mu\text{g MB-N g}^{-1}$ soil for P7S3 to a high of 96 $\mu\text{g MB-N g}^{-1}$ soil for P5S1 (Table 5.29). Compared to the other woolscour farm-soils, the absolute MB for the waterlogged soil of P7S3 was consistent with a reduction in MB of a stressed soil (Sparling, 1997). The MB estimated for P5S1 was more consistent with a healthy soil, which appeared to contradict management statements and the conclusions drawn from analysis for organic matter and N.

Normalising absolute MB-N by using the actual soil N, presented a different picture for MB (Table 5.29). When the amount of soil N immobilised in the MB (MB-N as % of soil N) is presented, P5S1 stands out as having a significantly lower MB than the other woolscour farm-soils. This conclusion was now consistent with the woolscour farm management's assertion that P5S1 was an unproductive paddock. The waterlogged soil (P7S3) also had a low MB-N (2.5 % of soil TN). However, based on comparison with other P7 samples (P7S1 and P7S2; Table 5.29), this was considered to be due to the waterlogged nature of P7S3, rather than the application of woolscour wastes.

When compared with the organic matter and MB levels of other woolscour-farm soils (P2, P7 and P10), P5 was atypical. It was therefore concluded that P5 represented soil that had been seriously compromised for nutrient cycling due to significant depletion of its MB brought about by the excessive application of woolscour sludge. Therefore, P2S2, P7S1, P7S2 and P10S1 were considered representatives of "normal" woolscour waste-amended soils and thus were suitable to use for comparison with other soil, either amended with woolscour wastes or not.

Table 5.29. Microbial biomass of woolscour farm soils (0-20 cm depth). Means are reported on an oven dry weight basis, n = 2.

Site	Flush-N ($\mu\text{g N g}^{-1}$ soil)	MB-N ($\mu\text{g MB-N g}^{-1}$ soil) ^a	$\frac{\text{MB-N}}{\text{TN}}$ (% of soil TN)
P2S2	24	68	3.78
P5S1	34	96	1.39
P7S1	25	72	4.80
P7S2	30	86	5.38
P7S3	18	50	2.50
P10S1	32	91	3.79

^a The K_{EN} value of 0.35 was used to convert flush N into MB-N was that of (Ross, 1992).

5.3.3 THE MICROBIAL BIOMASS OF THE “NORMAL” WOOLSCOUR-FARM SOILS

Parts of Tables 1 and 2 from (Brookes *et al.*, 1985b) are collated in Table 5.30 below. The data presented are from a range of agricultural soils on which the MB was determined using fumigation-extraction technique (only 0-23 cm depth are used) (Brookes *et al.*, 1985b), with the MB-N recalculated using k_{EN} of 0.35 (Ross, 1992) (Table 5.30). The MB-N ranged from a low of 1.9 % to a high of 9.0 % (average 4.8 % (of soil TN, n = 24; Table 5.30). The MB-N for “normal” woolscour farm-soils (P2S2, P7S1, P7S2 and P10S1) ranged from 3.8 % to 5.4 % (of soil N), with an average MB-N of 4.4 % (of soil TN), which was slightly lower than the agricultural soils in Table 5.30. Based on this comparison, the “normal” woolscour-farm soils appear to fall within an acceptable range for agricultural soils (Brookes *et al.*, 1985b), although a trend of slightly reduced MB is seen. P5S1 falls outside the entire range presented in Table 5.30.

The woolscour-farm soils can also be compared to the field soil amended with woolscour sludge during the experimental work in this thesis (Section 2.8.3). The MB of woolscour waste-treated soils averaged over five sites, which included P5S1 but omitted P7S3, which was considered stressed due to waterlogging, was 3.8 % (of soil TN), n = 5. This was 10 % lower than that for woolscour sludge-amended field soil, which had a MB of 4.2 % (of soil TN, n = 5; Table 3.22). This suggests that over the long-term, repeat applications of woolscour waste have the potential to depress the soil

MB. However the true impact can only be quantified by using a larger study, by including soils from many farms that have received woolscour wastes *and* with adequate non-amended reference soils (Brookes *et al.*, 1985b).

Table 5.30. Microbial biomass values for soils (0-23 cm) taken from Brookes *et al.* (1985, b). MB-N has been calculated ($k_{EN} = 0.35$) and $\frac{MB-N}{TN}$ (%).

History	pH	TN (%)	Flush-N ($\mu\text{g g}^{-1}$)	MB-N ($\mu\text{g g}^{-1}$)	MB-N (%) (of soil TN)
Park Grass (^{15}N -labelled N)	5.67	0.284	45.3	129.4	4.56
Park Grass (^{15}N -labelled N)	5.67	0.284	45.0	128.6	4.53
Park Grass (^{15}N -labelled N 1980)	5.67	0.284	38.7	110.6	3.89
Park Grass (^{15}N -labelled N 1981)	5.49	0.280	35.8	102.3	3.65
Park Grass (^{15}N -labelled N 1981)	5.49	0.280	36.1	102.3	3.65
Park Grass (^{15}N -labelled N)	5.49	0.280	38.9	111.1	3.97
Park Grass (^{15}N -labelled N)	5.61	0.206	39.8	113.7	5.52
Park Grass (^{15}N -labelled N)	5.61	0.206	39.8	113.7	5.52
Park Grass (^{15}N -labelled N)	5.61	0.206	39.8	113.7	5.52
Park Grass (^{15}N -labelled N)	5.60	0.204	43.1	123.1	6.03
Park Grass (^{15}N -labelled N)	5.60	0.204	44.5	127.1	6.23
Park Grass (^{15}N -labelled N)	5.60	0.204	41.1	117.4	5.75
Woburn (wheat, after arable rotation)	7.21	0.071	8.6	24.6	3.46
Woburn (wheat, after arable rotation)	7.21	0.071	4.7	13.4	1.89
Woburn (wheat, after arable rotation)	7.35	0.067	7.1	20.3	3.03
Woburn (wheat, after arable rotation)	7.35	0.067	7.1	20.3	3.03
Woburn (wheat, after 8 yr clover ley)	7.39	0.094	18.7	53.4	5.68

Woburn (wheat, after 8 yr clover ley)	7.39	0.094	29.6	84.6	9.00
Woburn (wheat after 8 yr ley)	7.12	0.102	9.3	26.6	2.61
Woburn (wheat after 8 yr ley)	7.12	0.102	19.7	56.3	5.52
Woburn (wheat, after 3 yr clover ley)	7.65	0.071	9.7	27.7	3.90
Woburn (wheat, after 3 yr clover ley)	7.65	0.071	15.9	45.4	6.39
Woburn (wheat, after 3 yr clover ley)	7.15	0.115	25.8	73.7	6.41
Woburn (wheat, after 3 yr clover ley)	7.15	0.115	18.4	52.6	4.57

5.4 CONCLUSION

In order to compensate for the lack of a control soil, the data manipulations above try to place the soils from the woolscour farm in context with other agricultural and woolscour sludge-amended soils. They are not intended to imply a degree of confidence in comparisons, as such confidence is not warranted. A general conclusion is that the woolscour-farm soils were not particularly different in terms of organic matter, TN or MB to other agricultural soils or other woolscour-amended soils, although a trend of decreased MB is seen. P5S1 was a consistent exception; this soil had been seriously and negatively affected by the dumping of woolscour wastes.

This, admittedly incomplete, case study shows that an estimation of the soil MB is an appropriate tool to use when collecting data that describes the environmental impact that land-based disposal of woolscour wastes may have. The case study presented data that were obtained independently from the woolscouring industry (apart from providing the access to a site), and places these data in the scientific arena. One of the strongest points of this case study is as a starting point from which other studies can be done. Land-based disposal of woolscour wastes may be better managed by including methodology similar to this case study's in a long-term monitoring scheme. Such an approach would allow woolscour wastes to be managed within the guidelines of Resource Management Act of 1991 (RMA).

Prior to the RMA in 1991, regional authorities set the amount of waste that could be disposed on land under the mandate of the Water and Soil Conservation Act of 1967

(WSCA), which lacked an emphasis on soil conservation seen in the RMA (Scott, 1992). Under the WSCA, councils were generally required to classify the minimum quality of a water body (that would be potentially affected by the waste disposal) to be *lower* than that at the time of the consent application. This meant that if a waterway were already polluted, say by a woollscour discharging effluent into the waterway or the run-off from land application of wastes entering it, rejection of a consent application was difficult and the waste disposal practice tended to continue as it had before consent application.

The RMA has, as one of its goals, the preservation of a resource, which must not be sacrificed to the goals of economic growth. This intent gives the RMA the flexibility to impose strategies that will improve soil and water compromised by previous mismanagement (Scott, 1992). However, for the RMA to be used to its potential reliable tools for determination of soil pollution must be available, and these tools need to be relatively quick and inexpensive. Chemical properties of the waste and the land to be used for waste disposal, present only part of the picture concerning the impact that land-based disposal of effluents may have on the environment. Biological properties are also needed, and I would argue that an appropriate tool to be used in the assessment of soil biological properties is the estimation of the soil MB. The MB is sensitive to soil management (Wardle, 1992) and a variation in MB assessments for potentially polluted soil was suggested in Section 4.5.3 above (Figure 3.41 (a, b)). Thus, the regular estimation of the MB on soil receiving domestic, agricultural and industrial effluents has significant benefits; it is sensitive of small and cumulative changes in soil, reproducible, inexpensive, and the results can usually be obtained within 3-4 days. Regional and District Councils should seriously consider adding this tool to their monitoring regime since it has potential to add significant data to the long-term effects that land applications of organic wastes may have on soils and thus promote the ideals embodied in the RMA.

6 CASE STUDY TWO: COMPOSTING OF WASTES FROM THE WOOL INDUSTRY

6.1 INTRODUCTION

The sheep and wool industry are attempting to reduce the quantity of waste that require disposal by non-sustainable methods such as landfill. Composting is a disposal method that has the potential to reduce the volume of organic waste material and concurrently produce a useful by-product (Section 1.3.1). Composting is currently being used by WRONZ for wastes from wool and textile industries. The project includes, amongst other wastes, wooll scour and fellmongery sludges. However, compost production *is* a waste management strategy, and as such will not be financially self-sustaining over the short-term. Rather it can help to reduce the gross cost of waste disposal, reduce the requirement for more landfill sites and improve business respectability.

This case study is an example of applied research and was an opportunity to include a small section of current industrial research using wooll scouring and fellmongering wastes. Even in its preliminary state (the project is anticipated to run for another four to six years), the composting project serves to indicate one approach that the wool and textile industries are taking to deal with their wastes.

6.2 MATERIAL AND METHODS

All decisions regarding the composting project, and all sampling of pre- and post-composted material, were made by WRONZ staff; including the waste materials used, the proportions of each mixed, the mixing process to produce different compost mixtures, the composting bin set-up, and the running and maintenance during composting. WRONZ provided samples of pre- and post-composted mixes for basic chemical analysis and a plant bioassay. Analysis of pre-composted mixes was done immediately (while composting was underway), and analysis and the bioassay of post-composted mixes were carried out concurrently when samples were received.

6.2.1 COMPOSTING SYSTEM

A forced-air static composting system was used, with air pumped in at the bottom of the composting bins. The rate of airflow was computer controlled and was a function of the internal temperature of the compost heap.

The components were added by volume (no weight data provided), which were intimately mixed in a large vessel until a homogeneous mixture was formed, this is termed "raw mix" (Table 6.31). The raw mixes were then used to fill 40-L plastic buckets (excess mix was used for initial analyses), which had been adapted at their base to permit aeration and drainage; all leachate was collected and returned to the surface of the mixtures.

The composting period was six weeks, after which time the temperature had returned to ambient. The material was removed from the compost bins and formed into windrows on polythene sheeting, and covered with polythene. A further six weeks of treatment under these "curing" conditions produced a material that was referred to as "composted mix". Total treatment time was 90 days.

Composting bins were designated HR (standing for HotRot™) 1 to 7, of Trial #1 or Trial #2, such that HR-3.1 was bin number 3 of Trial #1, and HR-7.2 was bin 7 from Trial #2. Pre-composted HR-mixtures are referred to as "raw" HR-mixes and post-composted HR-mixes are referred to as "composted" HR-mixes.

6.2.2 WASTE MIXTURES

The wastes came from woolscouring (sludges and wool fibre from the openers), fellmongering (sludge and skin trimmings), carpet production (fibre croppings from cut-pile carpet manufacture), sheep and deer skin production (wool-on skin, skin skin, dehaired-skin trimmings), and slaughterhouse tissue waste (udders, ears etc). Table 6.31 indicates the volume of waste materials and diluents used to produce the raw HR-mixes.

Table 6.31. Wool and sheep industry waste components used during two separate composting trials run by WRONZ, on a volume basis.

Waste	Waste Added (L)													
	Composting Trial #1							Composting Trial #2						
	1.1	2.1	3.1	4.1	5.1	6.1	7.1	1.2	2.2	3.2	4.2	5.2	6.2	7.2
Anaerobic WSc sludge	16	16	-	-	-	8	16	-	12	12	16	5	-	5
Settled WSc sludge	-	-	16	16	-	-	-	-	-	-	-	-	-	-
Carpet croppings	-	-	-	-	4	-	12	-	-	-	-	-	-	-
Opener waste	4	4	4	4	-	4	-	-	-	12	4	-	-	-
Deer/sheep skins	-	-	-	-	0.8	1.3	-	-	-	-	-	10	20	5
Fellmongery sludge	-	-	-	-	12	4	-	15	-	-	-	-	-	5
Wool-on-skin	-	-	-	-	-	-	-	-	-	-	-	10	-	-
Acid-cracked WSc sludge	-	-	-	-	-	-	-	-	-	-	-	-	10	-
Balance tank liquor	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Diluent	Diluent Added (L)													
Scoria	-	-	-	-	-	-	-	-	-	20	-	10	10	-
Pumice	-	-	-	-	-	-	-	-	30	-	-	-	-	15
Polystyrene	-	-	-	-	-	-	-	-	-	-	-	-	-	10
Polyester fleece	-	-	-	-	-	-	-	35	-	-	-	-	-	-
Sawdust	48	24	48	24	48	48	36	-	-	-	48	-	20	10
Compost	-	24	-	24	-	-	-	-	-	-	-	-	-	-
Supplement	Supplement Added (g)													
Super-phosphate	-	-	-	-	-	-	-	< 2	20	150	100	-	-	80

6.2.3 ANALYSES

Where possible, HR-mixes were broken up and sieved to <2 mm before chemical analyses, however most of the HR-mixes, especially from trial #2, could not be sieved to < 2 mm as they contained substrates that would not pass through a 5-mm sieve (Table

6.32). In these cases samples were used that were considered, visually, to be representative of the overall material.

Raw and composted HR-mixes were analysed for organic matter (Section 2.3.3), TN (Section 2.3.6) and mineral N (Section 2.3.7). Unless otherwise stated analyses were performed on duplicate samples and results are reported in an oven dry basis.

6.2.4 CUCUMBER BIOASSAY

Cucumber was used as the bioassay plant, based on the method of Chefetz *et al.* (1996). The suitability of composted HR-mixes as media for plant growth was tested in a glasshouse trial using seedling establishment and plant biomass as determinants.

6.2.4.1 Choice of Diluents

Most studies that investigate the suitability of certain substrates for composting use the final composted samples in undiluted and diluted states in plant bioassays to determine phytotoxicity (Zucconi *et al.*, 1981; Keeling *et al.*, 1994; Chefetz *et al.*, 1996; Pare *et al.*, 1997). Three diluents were used during the cucumber bioassay.

1. Ilam topsoil (TS). Topsoil was sieved to <5 mm prior to use. This diluent was selected to represent a probable domestic use of the composted HR-mixes, i.e. composts are usually mixed into garden soil and not used undiluted to grow plants. In its undiluted state, TS was a control for plant productivity in soil.
2. Seed raising mix (SRM). Seed raising mixes are media produced commercially and intended to enhance the germination of seeds and to aid in seedling establishment; they are available from normal retail outlets. The SRM used for the cucumber bioassay was "Black Magic" (a product by the horticultural firm Yates), which was used as purchased without analysis and its friable texture meant that sieving was not required as material was generally <5 mm. SRM was selected to provide the best medium available for germination of cucumber seeds and establishment of cucumber seedlings. In its undiluted state, SRM was a control for seed viability and seedling establishment, permitting (in theory) the maximum productivity of the bioassay to be determined.

3. Commercial compost (CC). Commercial composts are media produced commercially by composting of food, garden and manure wastes and are intended for incorporation into gardens to replenish nutrients and organic matter that have been lost through plant growth and removal of plant biomass. They are available from normal retail outlets. The CC used for the cucumber bioassay was "Soil Conditioner" (a product by Garden City Composts, which is part of the Christchurch City Council's recycling programme). It was used as purchased without analysis and its friable texture meant that sieving was not required, as material was $< 5\text{mm}$. This diluent was selected to provide material that was accepted, by commercial enterprises, as mature compost. Thus, undiluted CC permitted a direct comparison by which to judge the maturity of composted HR-mixes.

6.2.4.2 Dilution

Raw sludges (Table 6.31) were used undiluted or diluted to test their effect on cucumber seed germination; dilution was with an equal volume (20 cm^3) of TS or SRM.

Composted HR-mixes or CC were used undiluted or diluted to test their effect on cucumber seed germination and subsequent seedling establishment; dilution was with an equal volume (500 cm^3) of TS, SRM for Trial #1 and with SRM for Trial #2.

6.2.4.3 The Effect of Sludges on Cucumber Germination

There were three principal sludges used in HR-mixes; anaerobically digested woollscour sludge, settled woollscour sludge and fellmongery sludge. A fresh sample of each was provided by WRONZ for cucumber seed germination. Only the fellmongery sludge originated from the same operation as the fellmongery sludge used in the research reported elsewhere in this thesis (Section 2.2.2).

Approximately 30 cm^3 of diluted sludge, undiluted sludge, and the diluents were added to 9-cm Petri dishes and five cucumber seeds added. Plates (with lids) were placed in a glasshouse ($18 \pm 5\text{ }^\circ\text{C}$) and checked every day and moisture replenished as required. Treatments were in triplicate. Photographic evidence was used to determine

the effect that the sludges had on cucumber germination. Germination was considered complete when the first true leaves had emerged.

6.2.4.4 The Effect of Composted HR-Mixes on Cucumber Seedling Establishment

Approximately 750 cm³ of diluted sludge, undiluted sludge, and diluents were added to 1000-cm³ plastic pot-plant pots and 10 cucumber seeds planted. Pots were placed in a glasshouse (18 ± 5 °C), and were checked and watered every few days. Each pot was raised above the communal tray on top of another pot (turned up side down); this was to prevent absorption of leachates from other treatments. Pots were randomly moved relative to each other every 10 days to reduce the effect of light and temperature gradients. Treatments were in triplicate. Photographic evidence and above ground plant biomass (excluding TS data from Trial #1) were used to determine the effect that the composted HR-mixes had on the establishment of cucumber.

6.2.4.5 Bioassay Analyses

The bioassay was considered complete when control plants growing in SRM were approximately 40 cm high and had the fourth and fifth leaves emerging, which took 36 days for Trial #1 and 80 days for trial #2. The number of established seedlings and their biomass were recorded after 36 days for trial #1 and after 80 days for Trial #2.

Each replicate had ten seeds added, therefore, the potential to produce ten seedlings, which was scored as 100 % seedling establishment ($n = 3$). Dry-weight of all plants surviving at the termination of each bioassay was determined by oven drying at 50°C for 7 days. All plants *per replicate* were dried together and the average was taken for a treatment level ($n = 3$). The performance of the composted HR-mixes was evaluated by ANOVA for each trail, including the controls of CC and SRM. Composting Trial #1 and Trial #2 were not compared to each other.

6.3 RESULTS AND DISCUSSION

Both pre- and post-composted HR-mixes were unpleasant to work with and most became anaerobic when left to stand in their sampling buckets. In addition, most post-composted HR-mixes looked similar to their raw counterparts; added materials were readily recognisable (Figure 6.46). These observations indicated that the composted HR-mixes were *not* mature compost, in the accepted sense of the word. Thus, based on visual evidence the composted HR-mixes were not *compost*. The term “composted” will continue to be used in relation to HR-mixes that had received the composting treatment to clarify which HR-mix is being discussed.

6.3.1 CHEMICAL AND PHYSICAL PARAMETERS

Representative samples were used for all analyses, and where possible samples were sieved to < 2-mm, however this was not always possible (Figure 6.46). The material used for TN analysis was always < 2-mm, which was often a small proportion of the total sample (Table 6.32). To compensate for this “true” < 2-mm fraction, material that could be cut up and ground to pass through a 2-mm sieve was added to the true < 2-mm fraction. Hence subsamples, considered representative of the sample as a

Table 6.32. Particle size analysis of post-composted mixes from composting trial #2.

	Particle Size						
	(% of total sample)						
	HR-1.2	HR-2.2	HR-3.2	HR-4.2	HR-5.2	HR-6.2	HR-7.2
> 19 mm	24	0	17	0	61	45	5
>9.5 mm	39	53	59	0	34	37	31
> 7.0 mm	31	41	22	8	3	6	23
> 2 mm	4	4	1	63	1	5	21
< 2 mm	2	2	1	29	1	7	20

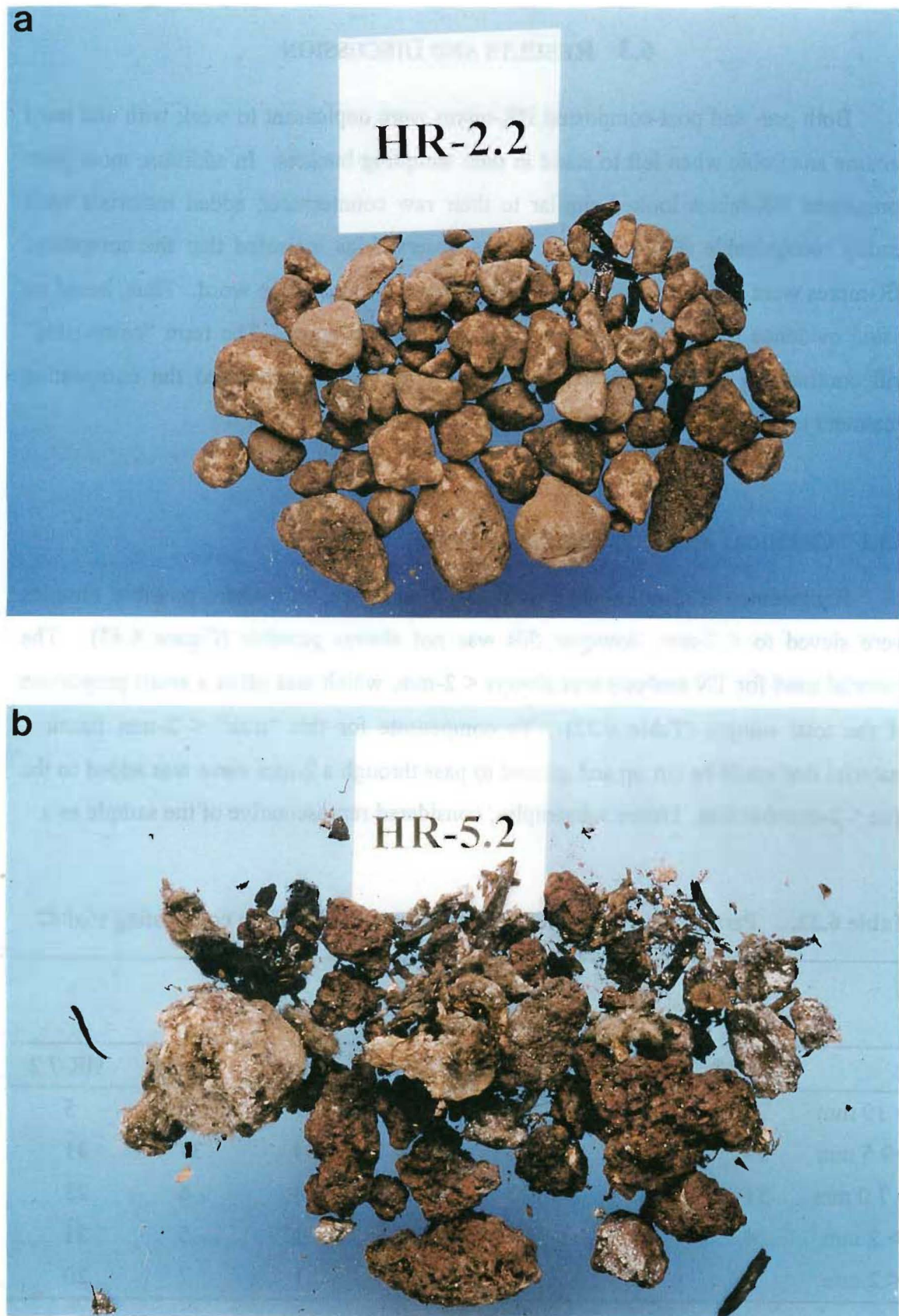


Figure 6.46. Post-compost treated HR-mixes, (a) HR-2.2 and (b) HR-5.2. Photos are essentially true to scale.

whole, were prepared and used for TN analysis. Ears, udders and skin pieces were the most difficult types of waste material to disperse evenly through the samples. Since these potentially contained most of the N, special care was taken to prepare finely divided samples that included representative proportions of these materials. For Trial #1, sawdust was the principal diluent and HR-mixes were, generally speaking, of a more even particle size than those of Trial #2 (personal observations; particle size analysis was not performed on Trial #1 samples). Particle size results for Trial #2 are in Table 6.32.

It is during the thermophilic phase of composting that significant reductions in organic matter occurs (Gray and Biddlestone, 1974; Anderson, 1990). However, for both Trials #1 and #2 the internal temperature of composting mixes did not exceed 45 °C, and was often not above 35 °C (personal communications WRONZ). Hence, the minimal reduction in organic matter seen for composted HR-mixes may have been due solely to the lack of a thermophilic phase (Table 6.33). Raw HR-mixes had an average of 70 % organic matter and the composted HR-mixes had an average of 66 %, over both trials.

Two HR-mixes stand out as being very low in organic matter, HR-2.2 and HR-3.2 (8 and 9 % organic matter respectively). Both these mixtures contained scoria and pumice as diluents, which made up most of the HR-mixture's weight, giving what are probably distorted organic-matter levels.

There was essentially no overall change in TN for raw vs composted HR-mixes (over both trials; Table 6.34). Raw HR-mixes had an average of 1.5 % TN and the composted HR-mixes had an average of 1.4 % TN, over both trials. While overall minimal change in TN occurred, individually half of the composted HR-mixes showed a decrease in TN, suggesting that N had been lost from the mixes by volatilisation (leachate was returned to mixes) during composting (Table 6.34).

Untreated mixes for composting usually have a C-to-N ratio between 30:1 and 50:1, which decreases during composting. Mature compost has a C-to-N ratio of 10:1 to 12:1 (Gray and Biddlestone, 1974; Atlas, 1988; Jenkinson, 1988; Chefetz *et al.*, 1996). Thus, composted material should have a TN (% dw) greater than the feed mix, due to the loss of C as CO₂ (thus giving a lower C-to-N ratio). If it were assumed that approximately half the organic matter of the HR-mixes was organic C, then the C-to-N

ratio of the raw and composted mixes can be calculated (Table 6.35). Only HR-6.1 showed a substantial decrease in the C-to-N ratio (decreased from 59 to 36), although this was still insufficient to qualify as composted material (Gray and Biddlestone, 1974; Chefetz *et al.*, 1996).

Table 6.33. Organic matter of raw and composted HR-mixes. Means (SE), n=2.

Organic Matter (%)			
	Ratio of Waste Components	Day 0 Pre-Composting	Day 90 Post-Composting
HR-1.1	80-20 WSc (AnO ₂)-Opener	93 (± 0)	93 (± 0)
HR-2.1	80-20 WSc (AnO ₂)-Opener	81 (± 0)	81 (± 0)
HR-3.1	80-20 WSc (Settled)-Opener	75 (± 0)	75 (± 1)
HR-4.1	80-20 WSc (Settled)-Opener	66 (± 1)	56 (± 5)
HR-5.1	24-5-71 Carpet Crop-Skins-Fell	93 (± 1)	90 (± 0)
HR-6.1	46-23-8-23 WSc (AnO ₂)-Opener-Skins-Fell	94 (± 1)	94 (± 0)
HR-7.1	57-43 WSc (AnO ₂)-Carpet Crop	89 (± 2)	90 (± 1)
HR-1.2	100 Fellmongery	97 (± 2)	94 (± 2)
HR-2.2	100 WSc (AnO ₂)	8 (± 0)	7 (± 0)
HR-3.2	50-50 WSc (AnO ₂)-Opener	9 (± 1)	3 (± 0)
HR-4.2	80-20 WSc (AnO ₂)-Opener	84 (± 4)	82 (± 6)
HR-5.2	20-40-40 WSc (AnO ₂)-Wool-skin-Skin	69 (± 7)	47 (± 8)
HR-6.2	67-33 Skin-WSc (AnO ₂ Acid Crack)	74 (± 3)	74 (± 1)
HR-7.2	33-33-33 WSc (AnO ₂)-Fell-Skin	45 (± 0)	37 (± 5)

The effect of composting on the production of mineral N in the composted HR-mixes varied considerably, some showing an increase in mineral N, others showing a decrease (Table 6.36). Overall, the raw HR-mixes had an average mineral N of 8.1 % (of the TN) and the average for the composted HR-mixes was 7.1 % (of the TN). With the exception of HR-6.1, all HR-mixes that included fellmongery sludge showed the largest increase in mineral N of any HR-mixes. HR-5.1 had an increase of 7.3 times the initial mineral N, HR-1.2 had an increase of 3.4 times, and HR-7.2 had an increase of 2.1 times.

Table 6.34. Total N of pre- and post-composted HR-mixes. Means (SE), n=2.

Total N (%)			
Ratio of Waste Components		Day 0 Pre-Composting	Day 90 Post-Composting
HR-1.1	80-20 WSc (AnO ₂)-Opener	0.91 (± 0.10)	0.71 (± 0.03)
HR-2.1	80-20 WSc (AnO ₂)-Opener	1.39 (± 0.06)	1.38 (± 0.09)
HR-3.1	80-20 WSc (Settled)-Opener	1.15 (± 0.01)	0.89 (± 0.07)
HR-4.1	80-20 WSc (Settled)-Opener	1.64 (± 0.03)	1.02 (± 0.38)
HR-5.1	24-5-71 Carpet Crop-Skins-Fell	1.51 (± 0.21)	1.34 (± 0.13)
HR-6.1	46-23-8-23 WSc (AnO ₂)-Opener-Skins-Fell	0.80 (± 0.04)	1.31 (± 0.29)
HR-7.1	57-43 WSc (AnO ₂)-Carpet Crop	2.20 (± 0.77)	1.95 (± 1.15)
HR-1.2	100 Fellmongery	1.29 (± 0.23)	1.48 (± 0.11)
HR-2.2	100 WSc (AnO ₂)	0.22 (± 0.02)	0.19 (± 0.02)
HR-3.2	50-50 WSc (AnO ₂)-Opener	1.52 (± 0.07)	0.31 (± 0.05)
HR-4.2	80-20 WSc(AnO ₂)-Opener	1.12 (± 0.08)	1.22 (± 0.08)
HR-5.2	20-40-40 WSc(AnO ₂)-Wool-skin-Skin	4.08 (± 0.51)	4.96 (± 1.13)
HR-6.2	67-33 Skin-WSc (AnO ₂ Acid Crack)	2.17 (± 0.45)	2.53 (± 0.07)
HR-7.2	33-33-33 WSc (AnO ₂)-Fell-Skin	1.46 (± 0.14)	0.85 (± 0.30)

Table 6.35. The C-to-N ratio of raw and composted HR-mixes.

Estimated C:N Ratio			
Ratio of Waste Components		Day 0 Pre-Composting	Day 90 Post-Composting
HR-1.1	80-20 WSc (AnO ₂)-Opener	51	65
HR-2.1	80-20 WSc (AnO ₂)-Opener	29	29
HR-3.1	80-20 WSc (Settled)-Opener	33	42
HR-4.1	80-20 WSc (Settled)-Opener	20	27
HR-5.1	24-5-71 Carpet Crop-Skins-Fell.	31	34
HR-6.1	46-23-8-23 WSc (AnO ₂)-Opener-Skins-Fell.	59	36
HR-7.1	57-43 WSc (AnO ₂)-Carpet Crop	20	23
HR-1.2	100 Fellmongery	38	32
HR-2.2	100 WSc (AnO ₂)	18	18
HR-3.2	50-50 WSc (AnO ₂)-Opener	3	5
HR-4.2	80-20 WSc(AnO ₂)-Opener	38	34
HR-5.2	20-40-40 WSc(AnO ₂)-Wool-skin-Skin	8	5
HR-6.2	67-33 Skin-WSc (AnO ₂ Acid Crack)	17	15
HR-7.2	33-33-33 WSc (AnO ₂)-Fell-Skin	15	22

Table 6.36. Mineral N (% TN) of pre-and post-composted HR-mixes. Means (SE), n=2).

Mineral N (% of total N)			
Ratio of Waste Components		Day 0 Pre-Composting	Day 90 Post-Composting
HR-1.1	80-20 WSc (AnO ₂)-Opener	14.30 (± 1.97)	3.23 (± 0.04)
HR-2.1	80-20 WSc (AnO ₂)-Opener	4.95 (± 0.21)	6.59 (± 0.30)
HR-3.1	80-20 WSc (Settled)-Opener	5.94 (± 0.09)	4.78 (± 0.19)
HR-4.1	80-20 WSc (Settled)-Opener	5.30 (± 0.13)	9.60 (± 0.01)
HR-5.1	24-5-71 Carpet Crop-Skins-Fell	2.01 (± 0.01)	14.58 (± 0.25)
HR-6.1	46-23-8-23 WSc (AnO ₂)-Opener-Skins-Fell	9.06 (± 0.74)	5.33 (± 0.17)
HR-7.1	57-43 WSc (AnO ₂)-Carpet Crop	8.21 (± 0.17)	1.51 (± 0.13)
HR-1.2	100 Fellmongery	4.35 (± 0.08)	14.82 (± 0.57)
HR-2.2	100 WSc (AnO ₂)	15.60 (± 0.56)	15.45 (± 1.50)
HR-3.2	50-50 WSc (AnO ₂)-Opener	4.77 (± 0.18)	1.54 (± 0.13)
HR-4.2	80-20 WSc(AnO ₂)-Opener	20.56 (± 0.24)	2.34 (± 0.15)
HR-5.2	20-40-40 WSc(AnO ₂)-Wool-skin-Skin	7.12 (± 0.08)	4.13 (± 1.48)
HR-6.2	67-33 Skin-WSc (AnO ₂ Acid Crack)	4.49 (± 0.08)	2.61 (± 0.30)
HR-7.2	33-33-33 WSc (AnO ₂)-Fell-Skin	6.30 (± 0.14)	13.10 (± 0.77)

This is consistent with the rapid mineralisation of N found during the decomposition of fellmongery sludge in microcosm experiments (Section 3.3.3). The only other samples to show an increase in mineral N were those with recycled compost as their diluents (HR-2.1 and HR-4.1, Table 6.36), suggesting that the MB in recycled compost was better adapted to the composting environment, thus allowing for a greater transformation of organic N.

Composted HR-6.1 was the only HR-mixture containing fellmongery sludge that did not show an increase in mineral N. HR-6.1 had a 64 % increase in TN and a decrease of 41 % in mineral N, suggesting immobilisation of N in the MB and was evidence that nutrient cycling was occurring. Most of the composted HR-mixes that showed a decrease in mineral N also had a decrease in TN, suggesting that at least some of the N had been lost *via* volatilisation or denitrification.

One must conclude from the work reported here that “composted” HR-mixes underwent little decomposition. Biological processes during composting may have been inhibited by heavy metals and pesticide residues, although sewage sludges (which

also contain these contaminants) have been successfully composted (Epstein *et al.*, 1976; Epstein *et al.*, 1978). As no quality control of the composting system was done prior to the addition of the wool industry wastes, physical problems with the composting system itself must be considered the most probable initial cause for the lack of composting. For example, a design or technical fault in the bin set-up, or maybe the lack of substrate mixing (during composting) caused an uneven distribution of moisture, air and/or nutrients. All, or any, of these situations would cause significant retardation of compost formation and may therefore explain the failure to produce compost in these two trials.

6.3.2 CUCUMBER BIOASSAY

The cucumber bioassay was set-up concurrently with the analysis of composted HR-mixes; hence the term “composted” will continue to be used to differentiate post-composting treatment, even though visual and chemical analysis indicated that the HR-mixes had not been composted.

The composted HR-mixes were used in a bioassay to determine whether they could be used as a suitable plant growth medium. Seed germination and seedling establishment are often used as an index in the determination of compost maturity, which is based upon the principle that immature compost inhibits of seed germination (Zucconi *et al.*, 1981; Keeling *et al.*, 1994; Chefetz *et al.*, 1996; Pare *et al.*, 1997).

6.3.2.1 The Effect of Sludges on Cucumber Germination

Three sludges were used as principal waste components of the HR-mixes, anaerobic woolscour sludge, settled woolscour sludge and fellmongery sludge. None of these sludges supported cucumber seed germination, even when diluted to half their strength with either TS or SRM (Figure 6.47 and Figure 6.48 (a)), whereas seeds germinated readily on SRM and TS (Figure 6.48 (b)). This suggested that the sludges contained, or produced, substances that were toxic/inhibitory to seed germination. This is consistent with inhibition of seed germination associated with organic matter under going rapid decomposition, due potentially, to release of inhibitory metabolites;

inhibition tends to decline rapidly as decomposition proceeds (Zucconi *et al.*, 1981; Chefetz *et al.*, 1996).

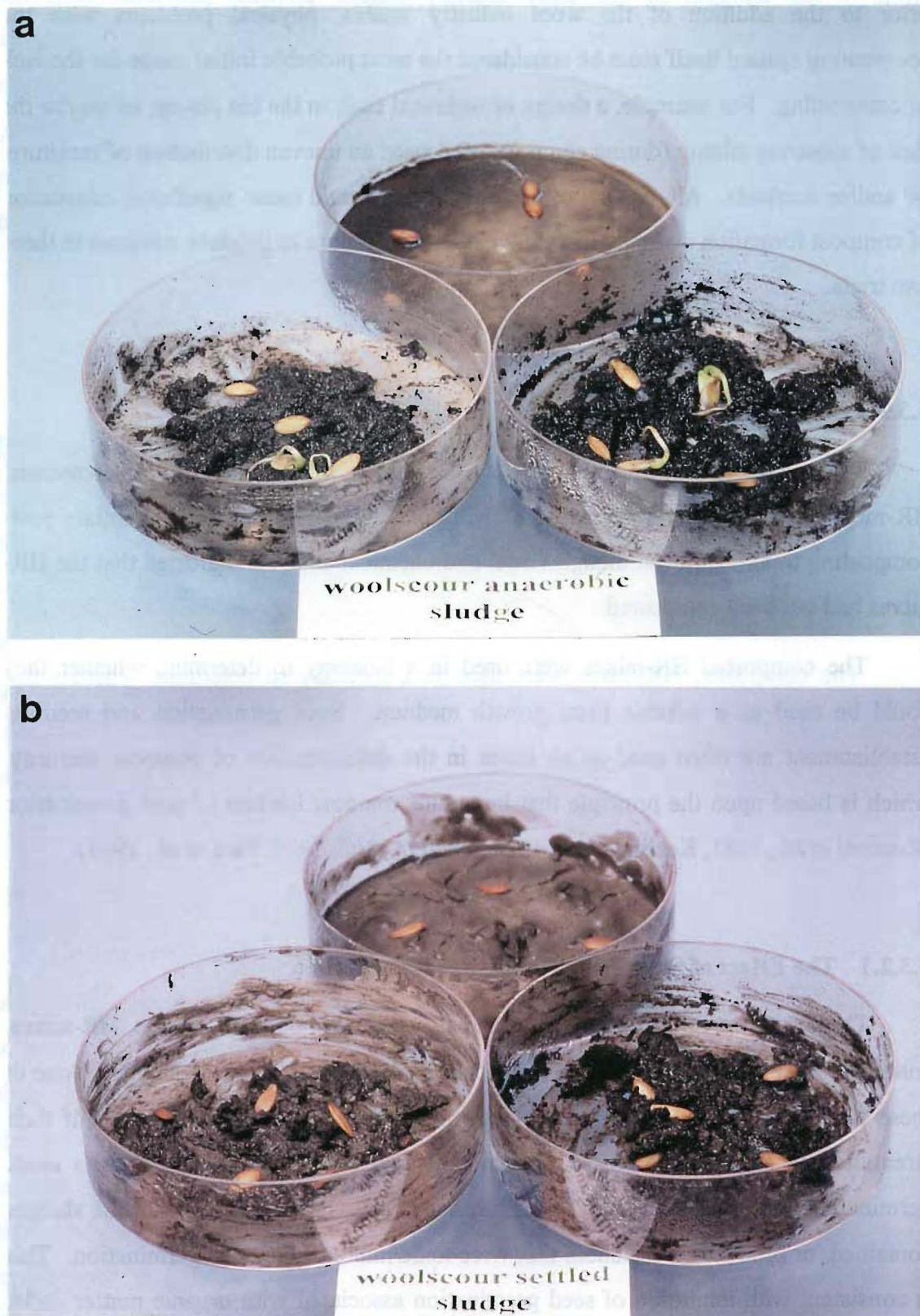


Figure 6.47. Cucumber seed germination (Trial #1) after 10 days on (a) Anaerobic woolscour sludge and (b) Settled woolscour sludge. Key: Back = 100 % sludge; Left = 50-50 sludge-TS; Right = 50-50 sludge-SRM. The Petri dishes were 9 cm in diameter.

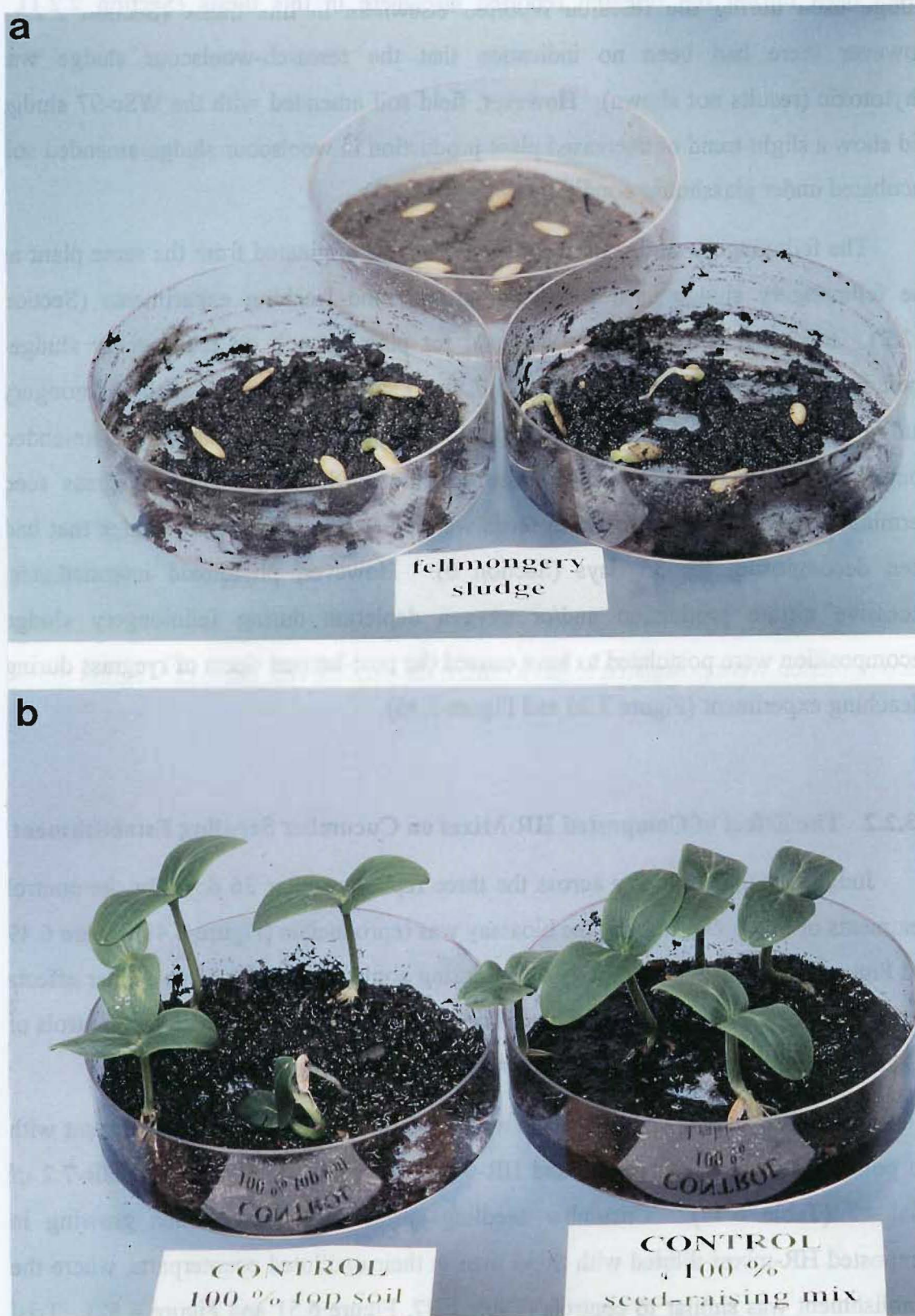


Figure 6.48. Cucumber seed germination (Trail #1) after 10 days on (a) Fellmongery sludge (Key: Back = 100 % sludge; Left = 50-50 sludge-TS; Right = 50-50 sludge-SRM. (b) Controls, Left = TS; Right = SRM. The Petri dishes were 9 cm in diameter.

The wooldscour sludges used for the HR-mixes were different from the wooldscour sludge used during the research reported elsewhere in this thesis (Section 2.2.1), however there had been no indication that the research-wooldscour sludge was phytotoxic (results not shown). However, field soil amended with the WSc-97 sludge did show a slight trend of decreased plant production in wooldscour sludge-amended soil incubated under glasshouse conditions (Section 3.7.3).

The fellmongery sludge used for the HR-mixes originated from the same plant as the fellmongery sludge used for decomposition and leaching experiments (Section 2.2.2). Inconsistent results were obtained for plant growth on fellmongery sludge-amended soil (Figure 3.37 and Section 3.7.2). Field soil amended with fellmongery sludge produced slightly more (non-significant) plant material than a non-amended control soil, incubated under glasshouse conditions (Section 3.7.2). Ryegrass seed germination was not inhibited when seeds were placed on fellmongery sludge that had been decomposing for 35 days. However, phytotoxic intermediates, excessive nitrate production and/or oxygen depletion during fellmongery sludge decomposition were postulated to have caused the post-harvest death of ryegrass during a leaching experiment (Figure 3.36 and Figure 3.37).

6.3.2.2 The Effect of Composted HR-Mixes on Cucumber Seedling Establishment

Judged by the similarity across the three replicates after 36 days for the control treatments of SRM, CC and TS, the bioassay was reproducible (Figure 6.49, Figure 6.50 and Figure 6.51). Photos were only taken during composting Trial #1 as similar effects were seen during Trial #2. Results from Trial #1 are compared only with the controls of CC and SRM that were run during Trial #1, similarly for Trial #2.

Most of the composted HR-mixes were unsuitable for seedling establishment with the possible exception of HR-5.1 and HR-6.1 of Trial #1 and HR-2.2 and HR-7.2 of Trial #2 (Table 6.37). Cucumber seedlings established better when growing in composted HR-mixes diluted with SRM than in their undiluted counterparts, where the establishment was similar to controls (Table 6.37, Figure 6.52 and Figure 6.53). Trial #2 had a significantly lower seedling establishment rate than Trial #1; 93 % seedlings established in Trial #1 on SRM, but only 67 % in Trial #2. This could have been due to the winter conditions of Trial #2, which would have had reduced light levels, even

though glasshouse temperature averaged 18 °C for Trial #2, or it could have been that the cucumber seeds had deteriorated during storage between trials. This stresses the importance of comparing within-experiment treatment to its within-experiment control, rather than between experiments.

In Trial #1 the CC supported cucumber seed establishment to a similar level as SRM (Table 6.37). However, as indicated in Figure 6.50, the plants growing on CC were not as vigorous as plants growing on SRM (Figure 6.49), after 36 days. This indicated that while CC and TS supported plant growth (Figure 6.50 and Figure 6.51), SRM was an ideal medium from a biomass production perspective and its inclusion as a control permitted maximum plant production to be determined.



Figure 6.49. The three replicates of cucumber seedlings after 36 days on seed-raising mix.

Table 6.37. Plants present in post-composted HR-mixes at the termination of bioassay. *100% = 10/10 plants survived. Means (SE), n=3.

Trial #1			
Plants established at termination of bioassay after 36 days			
(%)			
Ratio of Waste Components		Undiluted	Diluted 50-50 with SRM
HR-1.1	80-20 WSc (AnO ₂)-Opener	40 (± 0)	87 (± 13)
HR-2.1	80-20 WSc (AnO ₂)-Opener	73 (± 7)	87 (± 13)
HR-3.1	80-20 WSc (Settled)-Opener	20 (± 0)	100 (± 0)
HR-4.1	80-20 WSc (Settled)-Opener	17 (± 9)	97 (± 3)
HR-5.1	24-5-71 Carpet Crop-Skins-Fell	97 (± 3)	93 (± 3)
HR-6.1	46-23-8-23 WSc (AnO ₂)-Opener-Skins-Fell	93 (± 3)	97 (± 3)
HR-7.1	57-43 WSc (AnO ₂)-Carpet Crop	20 (± 0)	100 (± 0)
Commercial Compost Control (CC)		93 (± 3)	97 (± 3)
Seed-Raising Mix Control (SRM)		93 (± 3)	
Trial #2			
Plants established at termination of bioassay after 80 days			
(%)			
Ratio of Waste Components		Undiluted	Diluted 50-50 with SRM
HR-1.2	100 Fellmongery	23 (± 23)	83 (± 7)
HR-2.2	100 WSc (AnO ₂)	60 (± 15)	57 (± 12)
HR-3.2	50-50 WSc (AnO ₂)-Opener	17 (± 7)	67 (± 18)
HR-4.2	80-20 WSc(AnO ₂)-Opener	57 (± 9)	57 (± 15)
HR-5.2	20-40-40 WSc(AnO ₂)-Wool-skin-Skin	0 (± 0)	70 (± 12)
HR-6.2	67-33 Skin-WSc (AnO ₂ Acid Crack)	0 (± 0)	70 (± 6)
HR-7.2	33-33-33 WSc (AnO ₂)-Fell-Skin	60 (± 6)	70 (± 21)
Commercial Compost Control (CC)		37 (± 3)	60 (± 6)
Seed-Raising Mix Control (SRM)		67 (± 7)	

The differences seen in the size and height of plants growing in diluted composted HR-mixes, as opposed to the undiluted composted HR-mixes (Figure 6.52 and Figure 6.53), were reflected in the dry weight of the above ground biomass produced (Table 6.38). The dry weight of plants was used as the main parameter of the cucumber bioassay. Most composted HR-mixes produced less biomass than either CC or SRM (Table 6.38); HR-5.1, HR-2.2 and HR-7.2 were notable exceptions. The plant biomass

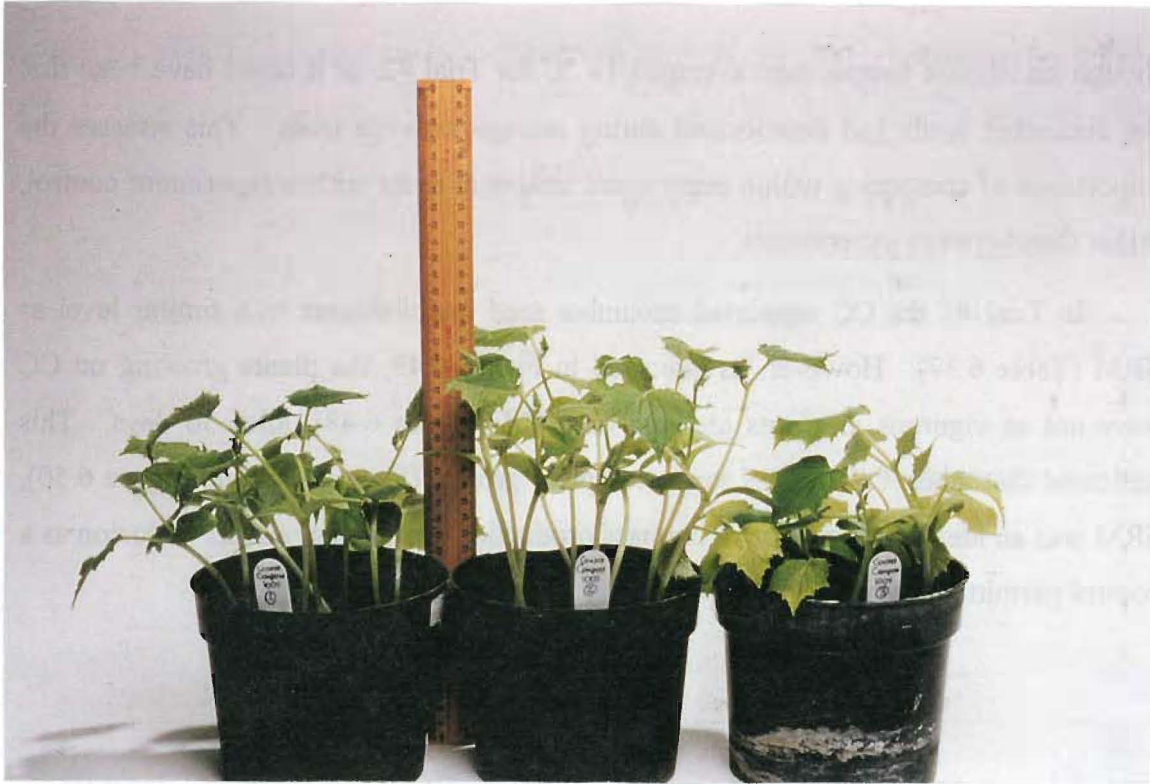


Figure 6.50. The three replicates of cucumber seedlings after 36 days on commercial compost.



Figure 6.51. The three replicates of cucumber seedlings after 36 days on topsoil.

produced from HR-5.1, HR-2.2, HR-4.2 and HR-7.2 was similar whether the composted HR-mix were diluted or not (Figure 6.52a, Figure 6.53). The two main comparisons to be considered were how much plant biomass was produced compared to the CC, and how much compared to the SRM control, within either Trial #1 or Trial #2.

Table 6.38. Dry weight of plants harvested from post-composted HR-mixes. Diluted HR-mixes were 50-50 HR-mix and SRM. Means (SE), n=3. ANOVA with Duncan's multiple-range test to compare means for undiluted mixes, cells with the different letters were significantly different ($\alpha = 0.05$).

Dry weight of plants at termination of bioassay Trial #1			
(g)			
Ratio of Waste Components		Undiluted	Diluted 50-50 with seed raising mix
HR-1.1	80-20 WSc (AnO ₂)-Opener	0.567 (± 0.06) e	4.755 (± 1.14)
HR-2.1	80-20 WSc (AnO ₂)-Opener	1.061 (± 0.11) d	5.421 (± 0.31)
HR-3.1	80-20 WSc (Settled)-Opener	0.233 (± 0.01) e	6.551 (± 0.39)
HR-4.1	80-20 WSc (Settled)-Opener	0.228 (± 0.11) e	5.332 (± 0.80)
HR-5.1	24-5-71 Carpet Crop-Skins-Fell	4.000 (± 0.11) b	5.569 (± 0.30)
HR-6.1	46-23-8-23 WSc (AnO ₂)-Opener-Skins-Fell	1.992 (± 0.29) c	6.404 (± 0.19)
HR-7.1	57-43 WSc (AnO ₂)-Carpet Crop	0.256 (± 0.02) e	5.574 (± 0.28)
	Commercial Compost Control	1.598 (± 0.14) c	8.702 (± 0.02)
	Seed-Raising Mix Control	7.065 (± 0.08) a	
Dry weight of plants at termination of bioassay Trial #2			
(g)			
Ratio of Waste Components		Undiluted HR-Mixes	Diluted HR-Mixes
HR-1.2	100 Fellmongery	0.117 (± 0.117) e	5.410 (± 0.272)
HR-2.2	100 WSc (AnO ₂)	6.026 (± 0.411) b	7.011 (± 0.642)
HR-3.2	50-50 WSc (AnO ₂)-Opener	3.946 (± 0.647) c,d	9.229 (± 0.944)
HR-4.2	80-20 WSc (AnO ₂)-Opener	3.827 (± 0.554) c,d	3.857 (± 1.342)
HR-5.2	20-40-40 WSc (AnO ₂)-Wool-skin-Skin	0.000 (± 0.000) e	6.561 (± 0.921)
HR-6.2	67-33 Skin-WSc (AnO ₂ Acid Crack)	0.000 (± 0.000) e	4.948 (± 0.583)
HR-7.2	33-33-33 WSc (AnO ₂)-Fell-Skin	10.459 (± 0.977) a	9.867 (± 1.680)
	Commercial Compost Control	3.493 (± 0.539) d	5.985 (± 0.522)
	Seed-Raising Mix Control	4.082 (± 0.142) c	

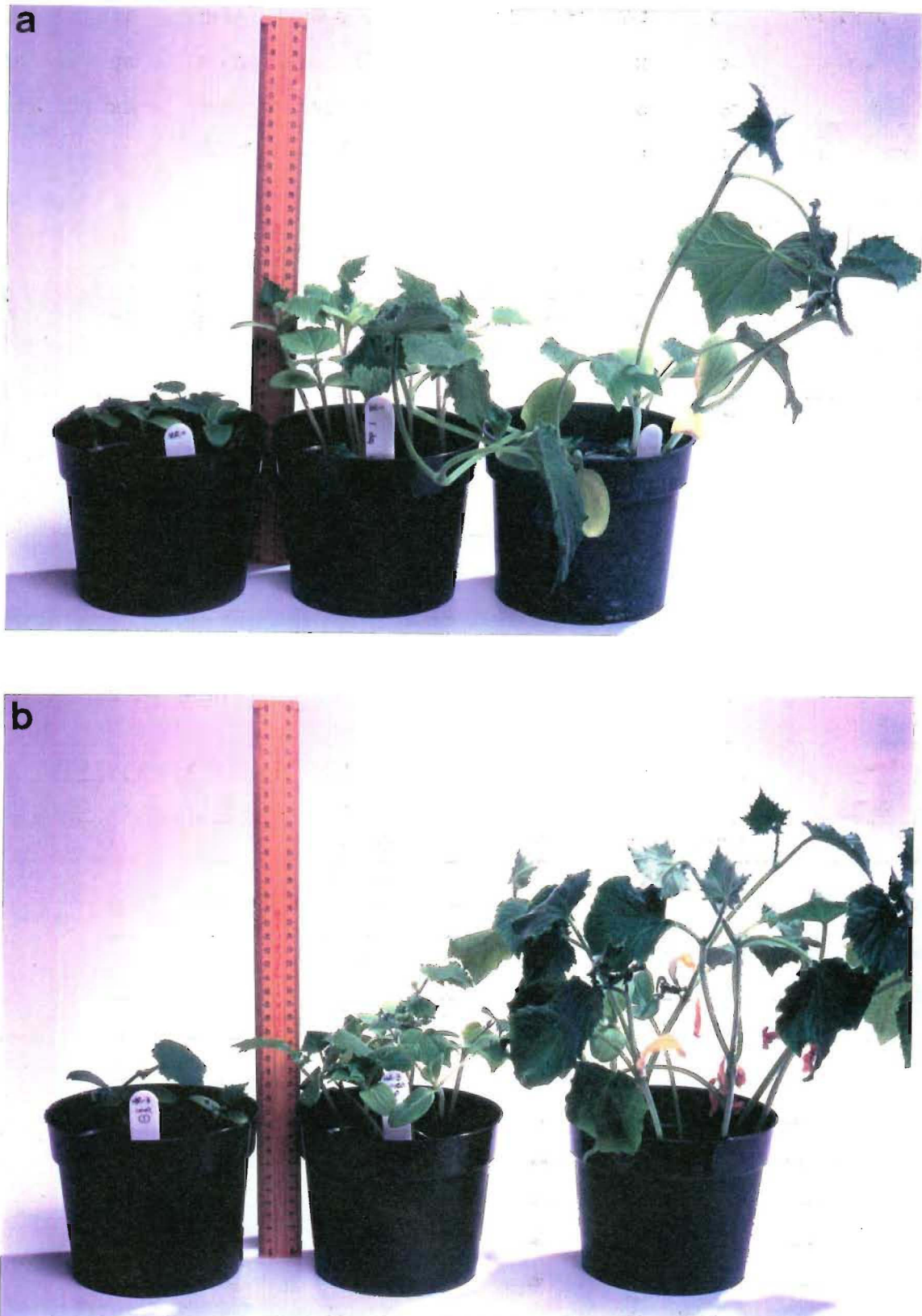


Figure 6.52. Cucumber seedlings after 36 days growth; Trial #1. (a) HR-1.1 and (b) HR-3.1. Key: From left to right: 100 % HR-compost; 50-50 HR-compost—TS; 50-50 HR-compost—SRM.

Trial #2 took place in winter, in a glasshouse, and ran for 80 days (*c.f.* 36 days for Trial #1), with the tallest plants about 40 cm for both trials. However, Trial #2 plants were more mature (than Trial #1) since most that were growing in the diluted treatments flowered. This illustrates the importance of running suitable controls for each trial and may explain the greater biomass produced from Trial #2 (most treatments).

In Trial #1 HR-5.1 produced significantly more plant biomass than any other compost, including the CC, but it still produced significantly less biomass than the SRM ($\alpha = 0.05$, Table 6.38). However, in Trial #2, HR-2.2 and HR-7.2 produced significantly more biomass than both SRM and CC. HR-5.1 and HR-7.2 both contained fellmongery sludge and showed an increase in mineral N due to composting treatment (Table 6.36), it was the abundance of mineral N in the medium that was considered to be primarily responsible for the better plant growth by these two HR-mixes.

HR-2.2 contained anaerobic woold scour sludge as the principal component, and although the level of mineral N did not alter due to composting, mineral N was also abundant in this HR-mix. The only post-composted HR-mix that had a high level of mineral N but produced low biomass was HR-1.2, which contained fellmongery sludge as its sole waste component. The reason for this apparent inconsistency may be that fellmongery sludge in HR-1.2 was still actively decomposing when used in the bioassay. Thus the depletion of oxygen, accumulation of toxic compounds from anaerobic metabolism or nitrate toxicity may have significantly affected plant productivity (Section 0).

6.4 CONCLUSION

Three lines of evidence were used to evaluate the effectiveness of composting wastes from the sheep and wool industries. Firstly, visual evidence of raw and compost-treated materials. Secondly, chemical and physical determination of several parameters associated with mature composts. Thirdly, evidence from the cucumber bioassay as to the suitability of compost-treated wastes for plant growth. Based on all three criteria, the compost-treated HR-mixes were not composted and failed to sustain healthy plant growth. The failure of waste materials to fully compost was considered

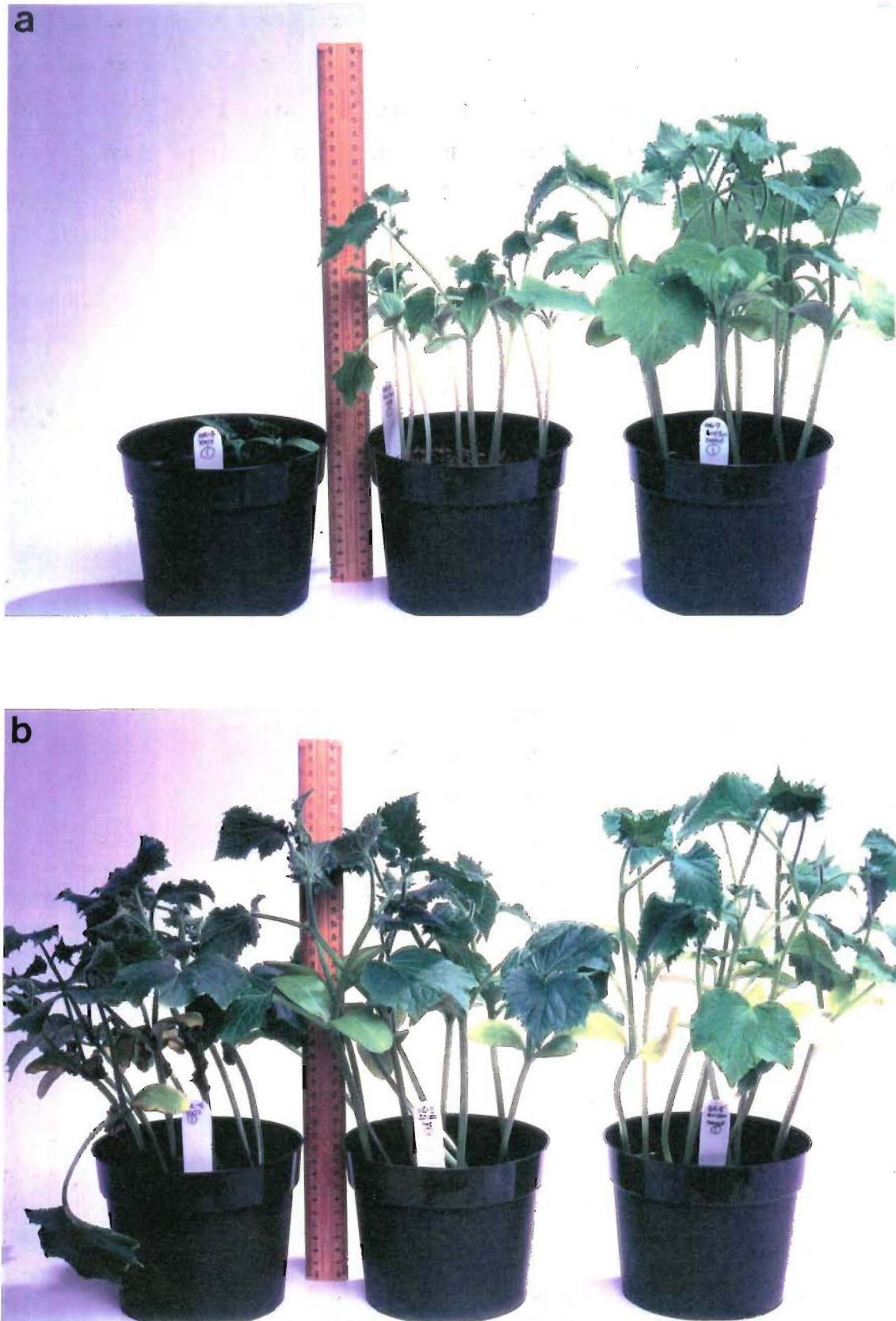


Figure 6.53. Cucumber seedling growth for Trial #1 after 36 days. (a) HR-7.1 and (b) HR-5.1. Key: From left to right: 100 % HR-compost; 50-50 HR-compost--TS; 50-50 HR-compost--SRM.

primarily due to technical/design faults in the composting system, rather than substrate quality or toxicity of the substrates. However, cucumber seedling growth could be used in future work as a reliable monitor of the extent of sludge decomposition.

7 CONCLUSION

The N content of organic waste material is frequently used as a principal factor to determine an application rate (kg N ha^{-1}) when the intended disposal method is on to agricultural land. The rationale being that as the waste decomposes it will provide N for crop uptake; i.e. the waste is being treated as a fertiliser amendment to soil. Rarely are data that describe the waste material's decomposition provided with other analytical data. Hence, an implicit assumption is made that different organic wastes decompose similarly and their N is equally available for mineralisation. The validity of this assumption must be seriously questioned.

Sludges produced by woolscouring and fellmongering industries are organic wastes that, because they are rich in N, are sometimes disposed of on agricultural land even though there are no biological data that indicate the extent to which they decompose. The aim of this thesis was to bridge this gap in knowledge by determining the decomposition of one woolscouring sludge and one fellmongering sludge. The hydrolysis of the woolscour and fellmongery sludge-organic N indicated that in both sludges the organic N was similarly distributed amongst the different forms of N and more than half was in the form of α -amino acid-N, which suggested that, the sludges were largely proteinaceous. Based on the N content of the woolscour and fellmongery sludges used here, hypothetically the woolscour sludge could be applied at twice the level of the fellmongery sludge in order to provide plants with equivalent amounts of N.

The central argument to this thesis is, therefore, that decomposition data are required to ensure the sustainable management of woolscour and fellmongery sludges, i.e. whether woolscour and fellmongery sludges can be used as fertilisers. To address the fertiliser value of woolscour and fellmongery sludges, three questions required answering. Firstly, the extent to which the sludges decompose, secondly, the constraints on sludge decomposition, and thirdly, the consequences of their decomposition in a soil system.

Each of the methods used to determine the extent to which the sludges decomposed, over medium-term incubations of approximately 50 days, indicated that woolscour sludge underwent significantly less decomposition than fellmongery sludge. The disparity between the sludges for the proportion of their N mineralised indicated that N availability was far lower from the woolscour sludge than the fellmongery

sludge. Therefore, even though on a chemical basis the α -amino acid-fraction in each sludge was similarly distributed, the decomposition data indicated that the organic N of woolsour and fellmongery sludges was not similarly available for microbial metabolism. Therefore, the first hypothesis stated in this thesis (H1 in Table 2.5) was not supported by experimental evidence, and it was concluded that the sludges were significantly different in their substrate quality.

If less N were mineralised from the woolsour sludge in the field, then the fertiliser value of the sludge would also be reduced. Thus, in theory, more woolsour sludge would need to be applied to meet the crop requirements for N. Applying woolsour sludge at such a hypothetical rate would mean that elevated amounts of Cd and Zn would be added to the soil. The presence of these metals, in excess, is likely to further reduce the amount of N mineralised; thus the N requirements of the plants would not be met by land application of the woolsour sludge in its current form.

The constraints upon the decomposition of fellmongery sludge indicated that it was decomposed by a principally aerobic and mesophilic microbial population and was thus sensitive to fluctuations in environmental conditions. Woolsour sludge decomposition appeared to be by a broader range of microbes, especially with respect to temperature. This suggested that two pools of soil organisms carried out the decomposition of woolsour sludge, one population with a mesophilic profile and one with facultative thermophilic representatives. Therefore, the second hypothesis of this thesis (H2 in Table 2.5) was not supported by the experimental evidence, and indicated that woolsour and fellmongery sludges will decompose differently under a variety of environmental conditions. This was further evidence that the quality of woolsour and fellmongery sludges was significantly different and assumptions made about the behaviour of one sludge would not, necessarily, be valid for the other sludge. For example, the suitability of woolsour and fellmongery sludges for disposal by land application or composting must be assessed in independent trials, since results based on one sludge can not be used to predict the behaviour of the other.

The weak depression of N mineralisation seen when fellmongery sludge was co-incubated with woolsour sludge or flower petals, although not considered significant at the replication level used, did suggest that nutrient cycling had been compromised. Further evidence that nutrient cycling was impeded by the presence of woolsour and fellmongery sludges was found when the sludges showed lower net-N mineralisation

when decomposing on previously amended soil. Therefore, the third hypothesis of the thesis was not supported (H3 in Table 2.5), indicating that wooldscour and fellmongery sludges were not neutral to nutrient cycling. The cumulative effect of reducing the turnover of organic matter would be the eventual loss of soil productivity for plant production. Therefore, the consequence of soil amendment with wooldscour and fellmongery sludges reducing nutrient cycling is serious and requires more work to better understand the implications of the results presented here.

The fourth hypothesis, that woolgrease would not inhibit the decomposition of proteins (H4 in Table 2.5), was supported by the work presented here, since casein-N was readily mineralised in the presence of woolgrease. It was concluded that the nature of woolgrease associated with wooldscour sludge was not a constraining factor on the decomposition of the sludge. The amendment of wooldscour sludge with readily available nutrient was relatively ineffectual at increasing net-N mineralisation, thus the fifth hypothesis was accepted (H5 in Table 2.5). However, the effect of Fe on wooldscour sludge decomposition suggested that there might be some benefit in amendment with this metal. This indicated that further work in this area is required to assess whether the Fe in wooldscour sludge is chelated and if so, how to release it for microbial metabolism. It was, therefore, concluded that the nature of the wool-N, present in the fibres of wooldscour sludge, was controlling the extent to which wooldscour sludge decomposed and mineralised N.

The decomposition of fellmongery sludge strongly indicted a resource of moderate-to-high substrate quality, and like sewage sludge, fellmongery sludge showed considerable potential to leach nitrate. Thus, H6 (Table 2.5) was rejected and it was concluded that one of the consequences of the decomposition of fellmongery sludge was the leaching of mineral N, especially nitrate. This indicated that extensive monitoring at sites of land disposal would be essential to ensure that nitrate pollution of waterways was minimised.

The amendment of soil with fellmongery sludge permitted more plant biomass to be produced, which indicated that fellmongery sludge had potential as a fertiliser. This evidence would, therefore, lead to the rejection of H7 (that similar amounts of plant biomass would be produced from a matrix amended with fellmongery sludge as an unamended matrix). However, some caution must be used if rejecting H7 outright, as cucumber seeds failed to germinate in the presence of fresh fellmongery sludge, and in

one experiment all the ryegrass on the amended columns died, while that on the non-amended columns survived. Therefore, longer-term studies and careful monitoring are required to assess whether these effects are likely to be seen on the field scale.

One of the consequences of applying woolscur and fellmongery sludge to soil was the reduction in the quantity of the MB; this was consistent across different scales. Therefore, H8 (Table 2.5) was not supported by the trends seen here and it was concluded that woolscur and fellmongery sludges do have the potential to negatively affect the soil MB and impede nutrient cycling. This conclusion is strongly supported by the decomposition of woolscur and fellmongery sludges in previously amended soils. The amended soils used came from the field study site and had a decreased MB relative to the non-amended soil, however they had not suffered the extensive depletion seen in the microcosm-scale soils. Therefore, the lower amount of N mineralised from the sludges in these soils indicated that the trend seen for the field soils (lower MB) was a real effect of soil amendment. On this basis, it is suggested that the slight trends of decreased N mineralisation seen when the sludges were mixed with other organic substrates in the microcosm studies, were real effects brought about by some factor(s), potentially common to both sludges, that restricted the functioning of the soil MB. The identity of this putative factor(s) was not determined during this experimental work. The sensitivity of the MB to changes in soil management, indicated that the MB of amended soils responded in a manner similar to the MB of non-amended soil, which suggested that H9 (Table 2.5) should be accepted. The response of the MB to changes in soil management also suggested that the determination of the MB from soils, incubated in microcosms at ambient temperature and moisture, might be a reasonable method to determine whether contaminated or compromised soils would respond to bioremediation. Such a method would potentially permit a series of small-scale experiments to be evaluated prior to the full remediation of sites.

Therefore, the woolscur and fellmongery sludges offered substrates of significantly different quality to microorganisms, such that, with all other conditions being equal, enzymatic attack required different subsets of the microbial community and that these organisms differed in their metabolic capacity. In addition, both woolscur and fellmongery sludges have the potential to decrease the soil MB and its ability to mineralise N. It seems reasonable to suggest that nutrient cycling would be

compromised by amending soil with woolscour and fellmongery sludges that are similar to the ones used during this research.

While the woolscour sludge used in this work is not currently disposed on to agricultural land, its disposal in landfill seems a waste of potential resources, yet based on the data presented in this thesis, land-disposal could not be recommended. This suggested that further investigations are required to determine a method of releasing the N within the woolscour sludge. Possible pre-treatments of woolscour sludge might be high temperature or solvent extraction to denature wool fibres. Both these conditions appeared to make the woolscour sludge-N more prone to microbial attack and therefore more likely to release N for plant uptake once applied to soil. On the other hand, fellmongery sludge, which currently is applied to agricultural land, does decompose readily and provides mineral N for plant growth, giving support to its use as a fertiliser. However, its propensity for nitrification in soil suggested that application rates and conditions must be carefully considered if pollution of waterways is to be avoided. The inhibition of seed germination and the death of ryegrass at the peak of sludge decomposition indicated that further work is required to more fully understand what caused these phenomena and whether they are a cause for concern during land-application.

This thesis also considered some applied aspects of the disposal of woolscour and fellmongery wastes. Two case studies, both incomplete by usual scientific criteria, provided data on the effect that woolscouring wastes might have on the soil MB and how the wool industry is trying to manage some of its wastes. In the first case study, there was an apparent depression of the MB in a soil that had received excessive woolscour sludges for at least 23 years, relative to soils that also received woolscour wastes but which were managed differently. This indicated that estimation of the MB would be a reasonable and sensitive tool to use when monitoring the impact that land-based disposal of effluents and sludges, such as woolscour wastes, was likely to have on the soil.

The second case study, which involved composting of wool-industry wastes by WRONZ, indicated that close attention to experimental set-up was required for applied research, since a conclusion could not be reached regarding the suitability of woolscour and fellmongery wastes for composting. The failure of woolscour and fellmongery wastes to degrade during composting treatment suggested that they were unsuitable

substrates for compost production. However, this conclusion was not consistent with the results in the main part of this thesis; the fellmongery sludge was a readily decomposable waste under aerobic and ambient conditions, and the decomposition of woollscour sludge responded well to high temperatures by showing increased N mineralisation.

The value of the two case studies included here was not just in the data accumulated, but also in the way that they highlighted the difficulties of independent and objective applied research of industrial wastes. Information provided by the different industries during the course of this thesis was sometimes inconsistent, and occasionally very misleading. For example, the manager of the woollscour operation from which the woollscour sludge was obtained indicated that the settling ponds were cleared every five to six months. However, an employee said that the settling ponds were dredged every two to three weeks depending on the amount of wool scoured. Without reliable information from industry it is exceptionally difficult to critically assess the overall integration of research presented in this thesis into the wider picture of waste disposal, since the actual amounts of wastes produced by the specific woollscour are not reliable. As no resource consent is required for landfill disposal of the woollscour sludge used here, there is no requirement for this woollscouring plant to be accountable for waste generation.

Another example of inconsistent information is highlighted by the WRONZ composting project. In the composting Trial #2 an "acid-cracked woollscour sludge" was used by WRONZ as one of the waste components (see Table 6.31). When enquiries were made to WRONZ into the quantity of this sludge produced in New Zealand, their reply was that "no acid-cracked sludges are produced in New Zealand".¹¹ It is difficult to reconcile the true composition of composting-project material provided for analysis and a bioassay with the selective information provided by WRONZ. Therefore, no information has been included in this thesis that quantifies the sludge(s)

¹¹ Personal communications between Dr L. G. Greenfield and WRONZ.

used as I consider such data to be unreliable and potentially grossly misleading, either for good or bad with respect to the industry.

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